THE UNIVERSITY OF ALBERTA

THE EFFECTS OF TEMPERATURE AND OXYGEN UPON THE LARVAL DEVELOPMENT OF *POLYMORPHUS MARILIS* VAN CLEAVE, 1939 IN *GAMMARUS LACUSTRIS* SARS

BY

© JOHN PETER EWANGE TOKESON

A THESIS

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ABSTRACT

The effects of temperature and oxygen on the development of Polymorphus marilis in Gammarus lacustris were studied in the field (at Cooking Lake, Alberta) and in the laboratory.

Cooking Lake is ice covered from late October or early November through late April; during most of this period, temperatures are stable at 1.0-1.5° C. Following freeze-up, dissolved oxygen levels decline rapidly, reaching zero by early January. In spring, temperatures and oxygen rise rapidly; the lake is thoroughly mixed by even light winds, and is essentially homogeneous with respect to temperature and oxygen. Temperatures reach the low twenties in July, then decline in August and September.

During the winter the prevalence of cystacanths is low; in the spring, the prevalence rises following the rise in temperature, and reaches a peak in June, after which the population is diluted by the new, uninfected generation of gammarids.

In the laboratory, an 80% reduction in the dissolved oxygen concentration of the water had no effect on the development of P. marilis. However, temperature was found to be extremely important. The threshold of development was found to be between 5 and 10° C. At temperatures of 5° C or lower the larvae were dormant; this dormancy could occur at any stage. Between 10 and 25° C, the logarithm of the speed of development was essentially linearly related to temperature. The speed of development at fluctuating temperatures, both above the threshold of development, was equivalent to that at a constant temperature of the same mean. When one temperature was below the threshold, all the

development occurred at the higher temperature, and at the normal rate. When infected gammarids were transferred to 23° C after 28 days at 10° C, development of the P. marilis was slower than normal; when transferred after 40 days at 10° C, development was faster than normal. These differences suggest a diapause condition.

Polymorphus marilis overwinters as hard-to-observe early stages, as indicated by the marked increases in the prevalence of observable larvae in overwintering gammarids collected from under the ice at Cooking Lake when the gammarids were incubated at 15° C or 23° C, but not when incubated at 5° C. The dormancy at temperatures of 5° C or less, and the diapause phenomenon are considered adaptations to ensure the overwintering of P. marilis as early larval stages in G. lacustris.

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INTRODUCTION

The acanthocephalan, Polymorphus marilis Van Cleave, 1939 (Palaeacanthocephala: Polymorphidae) is an important parasite of diving ducks of the genus Aythya and of various species of grebes in western Canada. It is abundant in Cooking Lake, Alberta, a major study area for parasitologists at the University of Alberta (Gallimore, 1964; Colbo, 1965; Graham, 1966; Denny, 1969; Podesta and Holmes, 1970a,b). Denny (1969) found that the amphipod, Gammarus lacustris Sars, served as the intermediate host of P. marilis in Cooking Lake.

Cooking Lake is a shallow, highly productive eutrophic lake, situated about 17 miles east of Edmonton, Alberta, covering an area of 35.12 km^2. Much of the lake is less than 6.5 feet deep, the maximum depth being about 10 feet. It is inhabited by a variety of invertebrates, birds, and mammals. Amphipods, Gammarus lacustris and Hyalella azteca, are the dominant larger invertebrates in the lake at all seasons. A variety of aquatic birds including scaup, Aythya affinis Eyton, and other waterfowl is common during the spring and summer months. Occasionally, muskrats and beavers are seen swimming in the lake. Kerekes (1965) and Denny (1967) have shown that temperatures vary with the season, being very low and uniform in winter and considerably higher in summer. The shallowness of the lake and the frequent winds make summer water temperatures homogeneous throughout the lake.

Dissolved oxygen concentration also varies with the seasons. In summer, the dissolved oxygen concentration is high, particularly during periods of algal bloom. During the winter, however, when the lake is completely ice-covered, oxygen concentrations dwindle until there is no more demonstrable oxygen in the lake in March. At this time of the year, the hydrogen sulphide concentrations are high.

In Cooking Lake, G. lacustris has an annual life cycle (Mennon, 1966; Denny, 1967). The overwintering adult males and females are in precopula before spring thaw. During and shortly after spring thaw, the males and females moult and copulate. During copulation, ovulation and fertilization occur (Mennon, 1966). The females carry their first brood in May. The number of eggs in each brood varies from 21-41, depending on the size of the gammarid. According to Mennon (1966), the eggs hatch and the young gammarids are released in the first week of June after an incubation period of three to four weeks. The water temperature at the same period during his study was about 15° C. After the first brood, surviving females may carry a second brood in July, Mennon (1966), although Denny (1967) did not observe a second brood. Mennon reported that the incubation period (2 weeks) for the second brood was shorter than that for the first; the water temperature at the later time was about 22° C. Obviously, the duration of the incubation period is influenced by temperature. Clemens (1950) found that the

incubation periods in Gammarus fasciatus varied from 7 days at a temperature of 24° C to 22 days at a temperature of 15° C.

The young are therefore recruited into the gammarid population yearly from mid-May to mid-July. They grow rapidly, developing secondary sexual characters (calceoli on the antennae in the males and rudiments of oostegites in the females) after attaining 7-8 mm in length (Denny, 1967). The calceoli in males are easily seen in July when the males from the first brood have reached 10 mm in length. The same length is reached by the females in August; at this time, the ovaries are visible through the integument as dark bands on each side of mid-dorsal line. At this stage, they are referred to as prereproductive adults (Mennon, 1966). They overwinter as such.

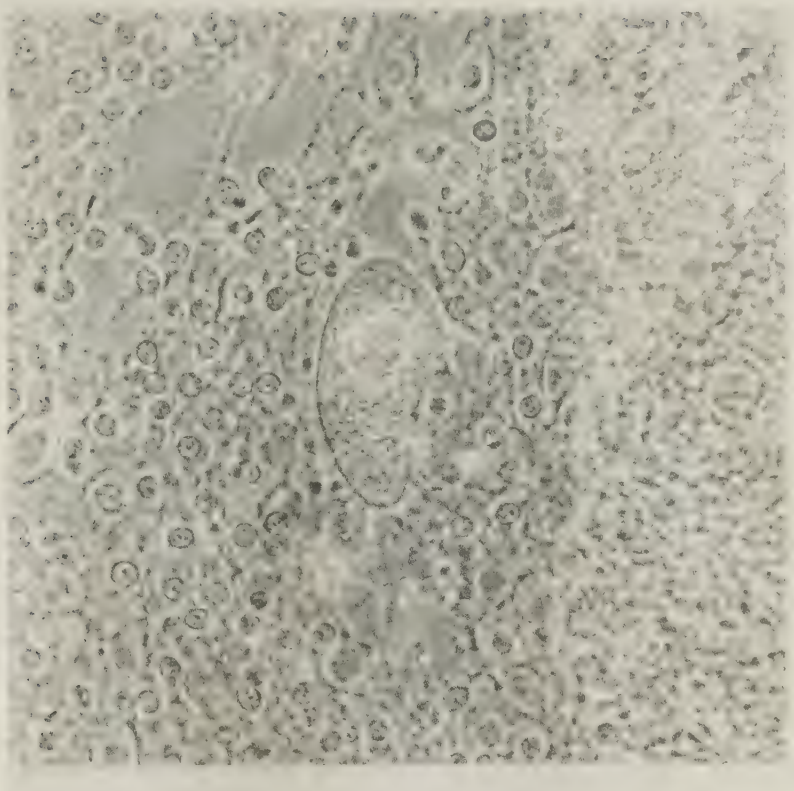
Towards the end of the recruitment period (mid-May to mid-July), the population reaches a maximum, with the young constituting 95% of the total population (Denny, 1967). After July, the population declines steadily. The abundance of the gammarid population in the fall is only about half of the maximum abundance in July. The decrease has been attributed by Mennon (1966) and Denny (1967) to heavy mortality of the young, which constitute 90% of the total population at freeze-up. During the winter, the abundance of the gammarid population is further decreased. There is heavy mortality, particularly in March, when the natural

environment is anoxic. A small percentage (about 10%) of the post-reproductive adults survive the winter, thus extending their life span to two years (Denny, 1967).

Development of P. marilis in G. lacustris is similar to that of other acanthocephalans in amphipods (Denny, 1969). Mature eggs released by gravid female worms, or the gravid female worms themselves, are passed with the faeces into the water. Gammarids become infected by ingesting the eggs scattered on the mud surface or with the female. The eggs hatch to release the acanthor which attaches to the gut wall, penetrates through the epithelial cells and lodges just inside the serosa of the gut.

The acanthor grows to a spherical body, bulging into the haemocoele, but still enclosed by the host serosa. This is the early acanthella stage (as defined by Hynes and Nicholas, 1957 and shown in Figure 1a). The acanthella continues to grow, bulging further into the haemocoele, forming a bladder-like structure still enclosed by the serosa. The serosa at the point of attachment constricts and finally pinches off. This is the mid-acanthella stage of Butterworth (1969) which is now free in the haemocoele. The acanthella in the haemocoele elongates to an elliptical shape. The giant nuclei, at this stage, are prominent, spherical and well established in the cortical syncytium. The proboscis is everted. This is the intermediate acanthella stage, shown in Figure 1b. As growth continues, the

Fig. 1a. Acanthor and early acanthella stages of Polymorphus marilis.
Acanthor: x 625; early acanthella x 250.



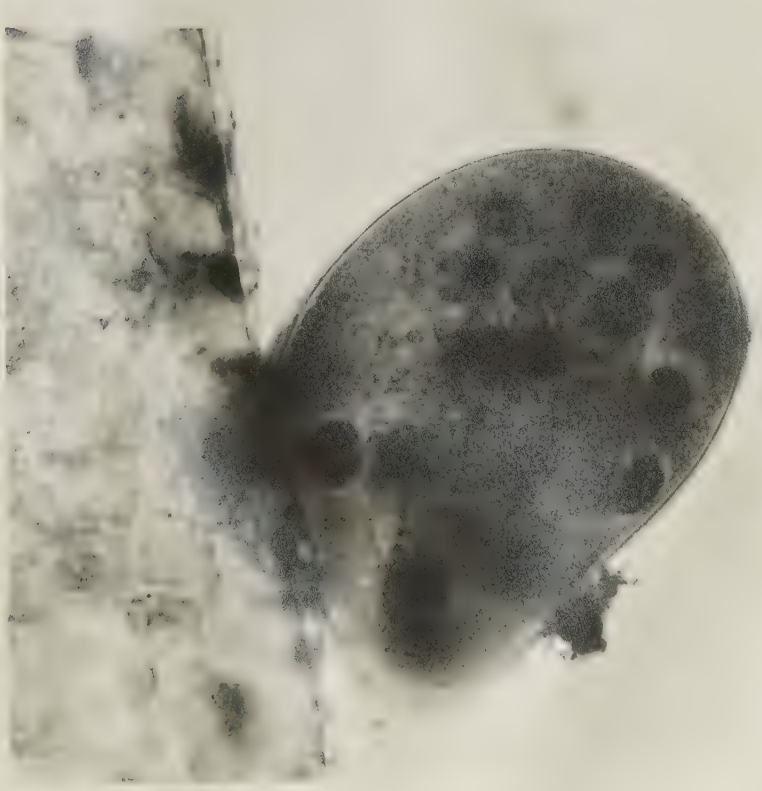
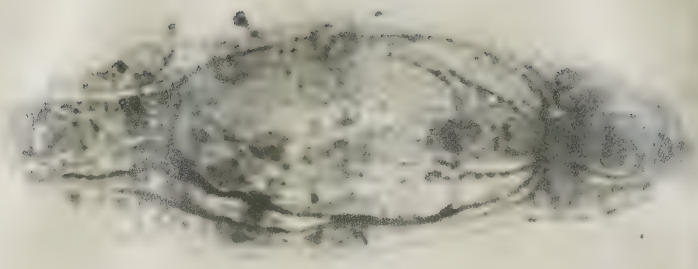
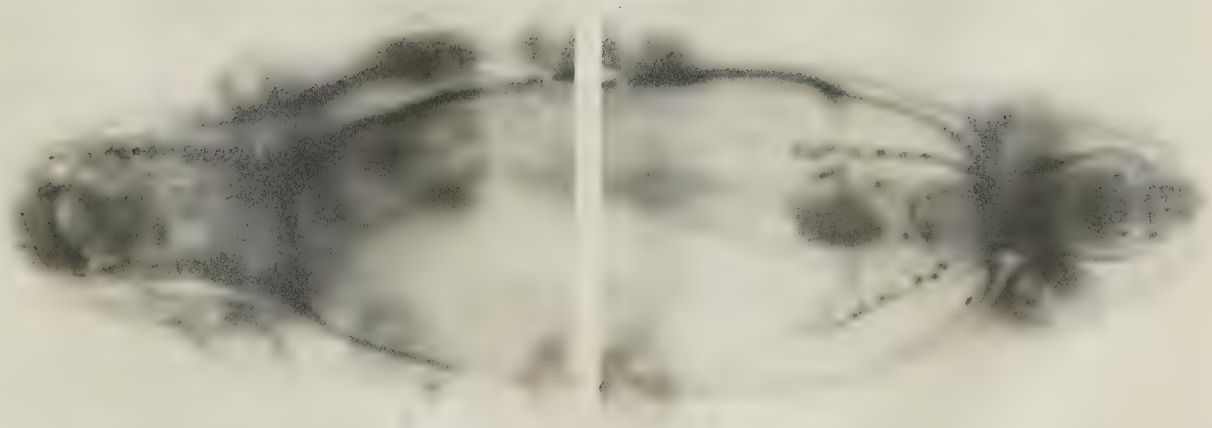


Fig. 1b. Intermediate acanthella, with enlargements of anterior (lower right) and posterior (lower left) ends. Intermediate acanthella (top) x 100. Anterior end (lower right) x 100; posterior end (lower left) x 100.





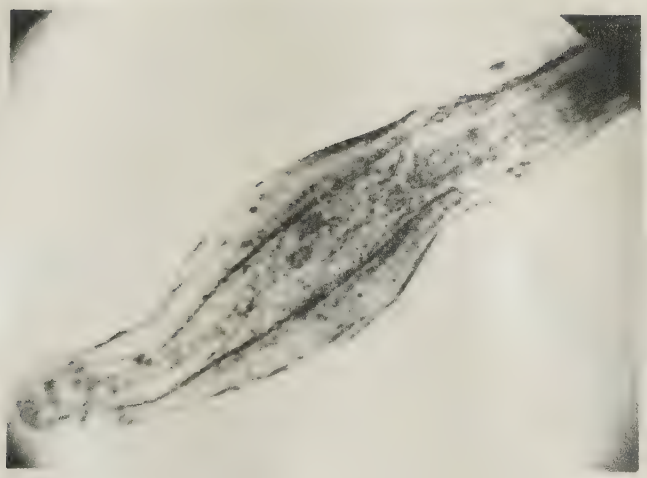
proboscis becomes inverted, the giant nuclei are no longer discernable, and the metasoma now becomes partitioned into anterior and posterior regions. The posterior region becomes thicker, the radial muscular layer of which is now easily visible. This stage is defined as the advanced acanthella stage, shown in Figure 1c.

With the completion of the development of the acanthella stages, the parasite encysts into the resting and infective stage; the cystacanth (Figure 1d). No further development occurs until the amphipod is eaten by the right definitive host.

Development of acanthocephalids in the arthropod intermediate host is known to be influenced by temperature. Temperature is recognized as an unquestionably important rate-controlling factor in the ecological and physiological processes of most animals, especially poikilotherms. Accounts of the various ways temperature may influence the development, distribution and abundance of organisms are well documented in Allee et al. (1949) and in Andrewartha and Birch (1954).

Although there has been growing interest in the influence of temperature upon the development of various parasites, there has been little or no detailed work done on the effect of temperature on acanthocephalids. Degiusti (1949) found that the development of Leptorhynchoides thecatus in Hyalella azteca was greatly retarded at

Fig. 1c. Advanced acanthella, with enlargements of anterior (lower right) and posterior (lower left) ends. The constriction of the metasoma is barely evident in this photograph. Advanced acanthella (top) x 85. Anterior end (lower right) x 85; posterior end (lower left) x 85.



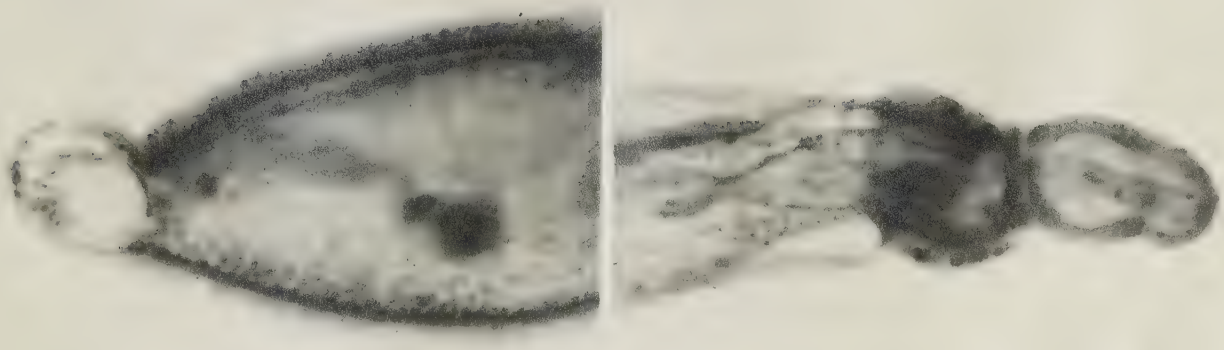


Fig. 1d. Cystacanth of Polymorphus marilis; x 200.

temperatures of 13 to 15° C; after two months post-infection the acanthella had grown to a size comparable to acanthellae eight days of age at 25° C. At temperatures of 20 to 25° C, it took thirty to thirty-two days post-infection to reach the infective stage. On the basis of such laboratory findings, Degiusti suggested that L. thecatus overwinters both as a larva within the amphipod intermediate host and as an egg free in the water.

Romanovsky (1964) in conjunction with his studies on the life cycle of Polymorphus minutus in Russia reported that P. minutus completed its larval development in 44 days at 16.4° C and 25 days at 24° C.

Awachie (1966) investigated the effect of temperature on the development of Echinorhynchus truttae in G. pulex and found that at 2-4° C (the normal stream temperature in winter) "only spherical and early larval stages were recovered from the shrimps after 136 days." At room temperature, these stages were normally reached after 20-24 days. He concluded that the development of E. truttae was drastically slowed under lowest winter temperature conditions.

Denny (1967) reported a minimal developmental time of 34 days for P. marilis in G. lacustris at 23° C. Denny examined large samples of gammarids collected from under the ice at Cooking Lake and found the acanthor stage of P. marilis more prevalent than any of the other larval

stages. When samples of gammarids collected from under the ice were incubated at a temperature of 22° C, he found an increase, first in the numbers of acanthellae, then in the numbers of cystacanths.

Olson and Pratt (1971) found that development of Echinorhynchus lageniformis in the amphipod, Corophium spinicorne, held at 11 and 13° C was immensely slower than those held at 23° C. They found that after 12 days at 11 to 13° C, the parasite was at a stage of development comparable to 1 to 2 days at 23° C. After 32 days post-infection at the lower temperature, the parasite developed to the acanthella similar to those found after 5 to 6 days at 23° C.

These studies indicate that the development of acanthocephalids is stemmed when the temperature is low, and suggest that they may very well overwinter as dormant early larval stages. They also indicate a rapid rate of development with increasing temperature.

It is against such a background that the present study was undertaken. The study is an attempt to answer the following questions:

1. Does P. marilis overwinter as a dormant early larval stage in G. lacustris?

2. Is dormancy in P. marilis a result of the effect of low temperature, low oxygen tension or a combination of both conditions?

3. Does increase in temperature result in rapid development and is there a direct, linear relationship between temperature and the speed of development of P. marilis in G. lacustris?

4. Is the speed of development of P. marilis in G. lacustris at fluctuating temperatures the same as the speed of development at a constant temperature equal to the mean of the fluctuating temperatures?

MATERIALS AND METHODS

Field Studies

Two environmental factors (water temperature and dissolved oxygen concentration) were studied quantitatively at an area of Cooking Lake known as "Diver Bay" (Figure 2).

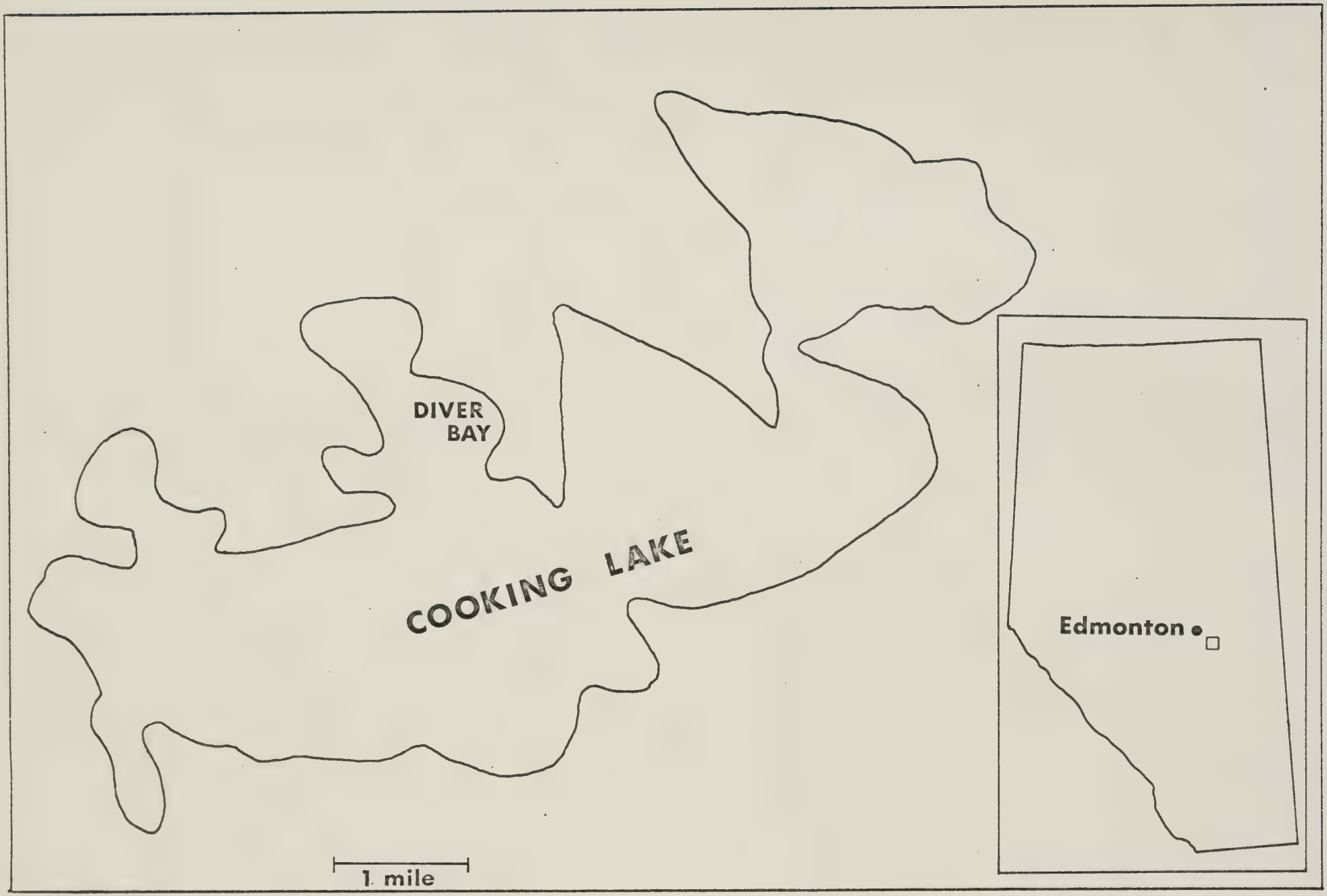
During 1968, water temperatures were recorded weekly in the summer and bi-weekly in the winter at different places of the study area. Recordings were made 15 yards offshore in about 12 inches of water and in the open lake, 200 yards offshore in about 3 feet of water, using a Yellow Springs Instrument tele-thermometer and a standard laboratory mercury thermometer. In the open lake, recordings were taken at the surface, and at the bottom.

In 1969, a Ryan thermograph stationed about 200 yards from the shore and about 15 inches beneath the water surface provided a continuous record of water temperature from May to August. A second thermograph was stationed 15 yards from the shore. Water temperatures were also taken periodically using the tele-thermometer and the laboratory thermometers used in 1968.

Dissolved oxygen concentrations were determined in replicated water samples taken at the same times and at the same sites as the water temperatures. Water samples were taken using a Kammerer bottle; analysis of the dissolved oxygen was performed using the standard Winkler technique.

During the winter months, water samples were taken

Fig. 2. Map of Cooking Lake, Alberta, showing location of Diver Bay.


DIVER
BAY
COOKING LAKE
1 mile
Edmonton

after cutting through the ice near the middle of Diver Bay with an ice auger. Care was taken not to disturb the water. Water temperatures were recorded immediately afterwards.

To determine the prevalence of cystacanths in gammarids, gammarids were collected weekly in summer and bi-weekly in winter in 1969 at the same times and sites as the temperatures and water samples were taken. While collecting gammarids from the open lake, the surface and the bottom were considered as one sampling site since the collecting technique employed could not adequately separate gammarids in the surface from those in the bottom. The technique required that the water be stirred vigorously before taking the samples thus making separation of surface from bottom gammarids impossible.

In summer, gammarids were collected by a dip net after swishing the net back and forth in the water in a random manner to ensure unbiased sampling. Gammarids were collected in winter by cutting through the ice with a chisel and spade, and again swishing the water back and forth and stirring vigorously before scooping up the top layers of the mud with a dip net. The gammarids were washed, conveyed to the laboratory and placed in aquaria. After stirring the water thoroughly, samples of 246 to 368 gammarids were withdrawn, examined and the number of cystacanths recovered was recorded.

Laboratory Studies

Maintenance of gammarids in the laboratory. Gammarids used in this study were maintained in continuously filtered dechlorinated tap water in aerated 10, 15 and 25-gallon aquaria with a thin layer of mud on the bottom. Garden soil, the source of the mud, was heated to prevent possible extraneous infections.

Vegetation (mainly Lemna trisulca) was included in the aquaria as a source of food and cover for the gammarids; lettuce provided a good food substitute. Once a week, gammarids were fed brewer's yeast.

Procedure in Infecting Gammarids

Uninfected gammarids to be used for the infection experiments were obtained in two ways. Female gammarids carrying embryos in the brood pouches were collected in the field and placed in 20 cm finger bowls in the laboratory until the young were released. The adults were discarded and the young transferred to stock aquaria. When such laboratory-raised gammarids were unavailable, gammarids collected in the field and kept in quarantine aquaria at 23° C for at least four weeks with no traces of natural infection were used. Four weeks at 23° C are ample time for an early infection (not immediately detectable) to develop to a stage which is readily visible through the gammarid cuticle.

Acanthocephalan eggs to be used for infecting gammarids were obtained from gravid female worms taken from naturally-infected scaup. The worms were teased apart in a drop or two of water on a glass slide. The eggs released from the body cavity were examined under a compound microscope. Eggs with mature acanthors (those with a central nuclear mass) were washed into finger bowls, about 6 cm in diameter, bringing up the water level to about 1 mm. About 30 grains of brewer's yeast were added to the finger bowl and mixed thoroughly with the eggs. Twenty gammarids were allowed to feed on the mixture for a period of one hour, after which the gammarids were removed, placed in a fine mesh net and washed thoroughly under running dechlorinated tap water to remove any eggs clinging to their tegument or appendages. After washing, the gammarids were placed in test aquaria.

Plan and Procedures of the Experiments

The questions this study is attempting to answer were investigated in six experiments in the laboratory.

The first experiment repeated Denny's (1967) work. Gammarids collected from under the ice at Cooking Lake were brought into the laboratory and were divided into three separate lots of 2,800 animals per lot. Three hundred gammarids from each lot were autopsied immediately to determine the prevalence and stage of development of *P. marilis*.

The remaining gammarids from each lot were divided into groups of 500 and transferred to each of five experimental aquaria maintained in incubators kept at three constant temperatures (5°, 15°, and 23° C). Thirty-five to fifty gammarids from each aquarium were examined every 2 weeks to determine the stage of development of the parasite. Dissolved oxygen concentrations of the water in the aquaria were determined using Burke's (1962) micro-Winkler technique.

The second experiment was designed to determine the growth rate of P. marilis under controlled, constant conditions. One thousand, eight hundred gammarids were infected in the laboratory and divided into 6 lots of 300 gammarids each. One of the lots was placed in an aquarium maintained at each of the following constant temperatures: 5°, 10°, 15°, 20°, 23° and 25° C, in incubators or controlled temperature rooms. This experiment ran for 160 days. In the early days of the experiment, 40-50 gammarids were examined every 1-20 days; later, when fewer gammarids were present, only 20-35 gammarids were examined at each time. The concentration of dissolved oxygen in the experimental aquaria was determined every other day by Burke's (1962) micro-Winkler method. The experiment was replicated five times.

The third experiment was designed to determine the rate of development of P. marilis under fluctuating temperature conditions. Two thousand, seven hundred laboratory infected gammarids were divided randomly into 9 lots of 300 gammarids

Table 1. Dissolved oxygen content in cultures during the development of the larval stages at the different constant temperatures.

Temperature	Dissolved oxygen in ppm mean	range
5	11.3	11.0-11.6
10	9.1	8.9- 9.4
15	8.5	8.4- 8.8
20	7.6	7.3- 8.1
23	7.3	7.2- 7.6
25	6.4	6.2- 6.8

each. The lots were placed in aquaria, assigned the letters A, B, C, D, E, F, G, H, or J, and incubated in incubators or controlled temperature rooms at the constant temperatures: 5°, 15°, 20°, 23°and 25° C in the following manner: A and B were maintained at 5° C, C and D at 15° C, E and F at 23° C, G and H at 25° C, J at 20° C. Lots A, C, E, G, and J were used as controls; lots D and H were alternated every 48 hours between 15° and 25° C, both of which were above the threshold temperature of development (D was first incubated at 15° while H was first incubated at 25° C). Lots B and F were alternated between 5° and 23° C, 5° C being below the threshold temperature of development and 23° C above it. (Threshold temperature of development as defined by Peairs (1927) is the temperature above which initial development commences or below which development is discontinued.) The experimental aquaria were transferred suddenly from one temperature to another, but some time elapsed before the water in the aquaria cooled down or warmed up to the second temperature. Analysis of dissolved oxygen concentration in each aquarium was done every other day, using Burke's (1962) micro-Winkler technique. Gammarids (50 or sometimes 20 to 30 in number) were withdrawn periodically from each aquarium and examined. The experiment was repeated four times.

The fourth experiment was designed to test the effects of prolonged incubation at 5° C. Two thousand, four hundred laboratory-infected gammarids were divided into 6 experi-

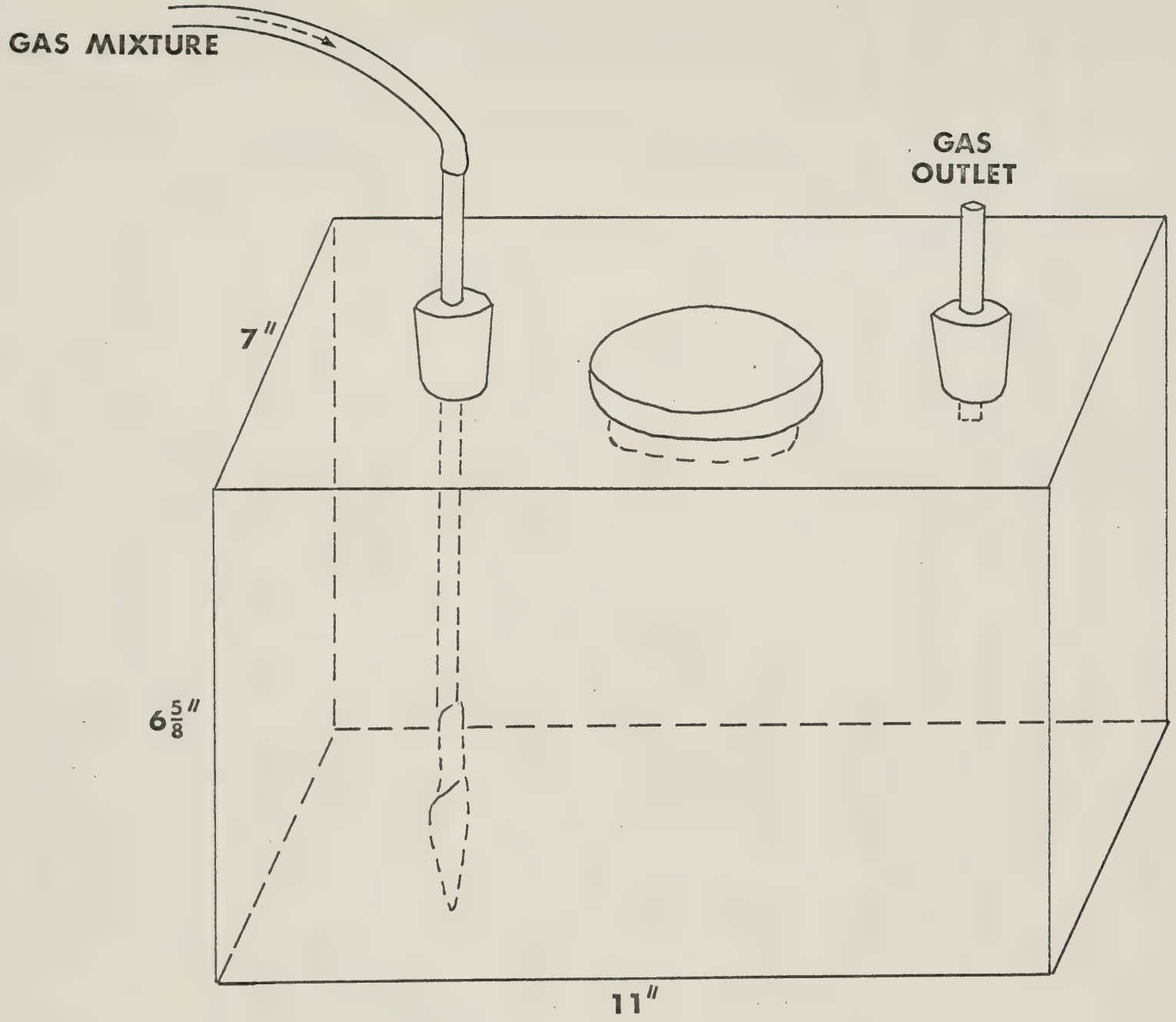
mental and 2 control lots averaging 300 gammarids per lot. Control lots were incubated at 5° C and 23° C. Three experimental lots (A, B and C), maintained at 23° C were transferred to 5° C after the development of P. marilis in the gammarids had reached different acanthella stages. A was transferred after the parasite had developed to the early acanthella stage (day 10), B after it attained the intermediate acanthella stage (day 18), and C after it attained the advanced acanthella stage (day 23). Three other experimental lots (D, E, and F), maintained at 5° C, were transferred to 23° C on days 10, 18 and 23 post-infection. Gammarids were periodically withdrawn from experimental and control lots, both before and after transfer, and examined. The dissolved oxygen concentration in the water in each aquarium was analyzed by Burke's (1962) micro-Winkler technique. The experiment was repeated five times.

The fifth experiment was designed to observe the effect of partial development of P. marilis at 10° C on the subsequent development at 23° C. Laboratory infected gammarids were portioned randomly into 8 lots of 400 gammarids per lot. Two lots, one maintained at 10° C, the other at 23° C, were used as controls. Of the remaining six lots, three (A, B, and C) were maintained at 10° C for 28 days before transfer to 23° C; the others (D, E, and F) were maintained at 10° C for 40 days before transfer to 23° C. One hundred gammarids were withdrawn from each experimental lot and

examined on days 28 and 40 respectively before transfer. Periodically after transfer, 30-50 gammarids (depending on the number still alive in the aquaria) were withdrawn and examined. Gammarids from the control lots were examined at the same times. Dissolved oxygen analysis was carried out every other day by Burke's (1962) micro-Winkler technique.

The sixth experiment was designed to determine the effect of reduced oxygen concentration on the larval development of P. marilis. Special tanks (Figure 3) were designed for this purpose. Water to be used in the tanks was made anoxic by passing a stream of pure nitrogen through it for an hour or until no traces of oxygen could be detected by the micro-Winkler technique. The water was then transferred to six experimental tanks; three tanks were aerated with a mixture of 90% N_2 and 10% O_2 and the other three with a mixture of 97% N_2 and 3% O_2. A total of 160 laboratory infected gammarids were placed in the tanks at each gas regime. The tanks were maintained at 23° C in controlled temperature rooms. Routine examination of the gammarids was carried out periodically to determine the stage of development of the parasites. The concentration of dissolved oxygen in the water was determined every other day using Burke's (1962) micro-Winkler technique. Water in the tanks was replenished when necessary using water aerated with the same gas mixture.

Fig. 3. Sealed aquarium for studying the effects of low oxygen tension.


GAS MIXTURE
GAS
OUTLET
7″
6 5/8″
11″

Procedure for the Examination of Gammarids

The gammarid to be examined was transferred to a drop of water on a glass slide under a dissecting microscope; severed at the level of the third segment from the posterior; the cuticle, but not the gut, was dissected loose behind the head; and the head was pulled gently, carrying with it the digestive glands and tract freed from the body cuticle. The digestive glands and tract were covered with a cover-slip sealed with vaseline to prevent drying. The slide was transferred to a compound microscope to observe and count the early acanthella stages.

For the observation of the later developmental stages, the same method of dissection was adopted. However, in these cases, the larvae popped out into the fluid on the slide as soon as the gammarid was cut open. The number of larvae in each stage in each gammarid dissected was counted. For each sampling period, the data obtained from all the gammarids examined from each lot were pooled and the percentage of each stage was determined.

The T_{50} (time required for 50% of the larvae to reach a particular developmental stage) was used as the criterion of development. The exact T_{50} could not be determined, since the developmental stages of the larvae could not be distinguished without killing the amphipods. Since the numbers of infected gammarids, and facilities for keeping them, were limited, the number of observations that could

be made was also limited. The intervals chosen were those suggested by the results of preliminary experiments.

Because of these difficulties, the T_{50} was determined by interpolation, assuming a linear relationship between time and proportion of larvae reaching the next stage. For example, 20.5% of the larvae had reached the intermediate acanthella stage on day 35 post-infection and 94.8% on day 40. Graphic interpolation gave a T_{50} of 36.99 days. In some cases, the T_{50} had to be extrapolated from two sets of data above (or below) 50%.

RESULTS

Field Studies

The data obtained on water temperatures and dissolved oxygen concentrations during 1968 and 1969 are shown in Figure 4. Values shown for oxygen concentration and for temperatures are the monthly means of measurements made at the shore station (15 yards offshore), where the majority of the infected gammarids were observed, during visits to the lake.

Both years were characterized by similar patterns in the temperatures and dissolved oxygen concentrations. In winter, when the lake was completely ice-covered, water temperatures taken under the ice were fairly uniform. They declined from about 4° C at freeze-up to a minimum of less than 1° C in January, 1969, then warmed up slightly to about 1.5° C in March, a month before break-up. In the winter of 1969-1970, temperatures had dropped to 1° C in January when observations ceased. Oxygen concentrations similarly declined from 3.0 ppm in November 1968, and 6.6 ppm in November 1969 to 0.0 ppm by the following January. When the dissolved oxygen concentration fell, the H_2S concentration rose, reaching levels as high as 5.0 ppm from March through early April in 1969.

At break-up in April (when the offshore part of the lake was still ice-covered) differences in water temperatures were observed between locations. In 1968 the water

temperature in open water about 30 feet offshore was 8.5° C, at the same time, the temperature under the ice, about 200 yards offshore, was 2.0° C. In 1969, the temperature of the open water 30 feet offshore was 11.6° C and that under the ice, 200 yards offshore, was 1.5° C. Oxygen concentrations exhibited similar differences between locations. In 1968, the dissolved oxygen concentration of the open water was 8.0 ppm; under the ice, 200 yards offshore, it was 1.2 ppm. In 1969 the values for dissolved oxygen concentration were 8.5 ppm in the open water and 1.8 ppm under the ice. However, those break-up conditions are short-lived, lasting only from one to two weeks.

In the summers of 1968 and 1969 the water temperatures showed marked seasonal patterns. In 1968, the monthly mean temperatures were highest in July for all locations. Values were as high as 20.2° C at 15 yards offshore in about 12 inches of water; at 200 yards offshore values were as high as 20.6° C in about 15inches of water and 18.1° C in about 3 feet of water. In August, water temperatures began to drop at all the locations, reaching as low as 5.3° C at 15 yards offshore in October, at 200 yards offshore, temperatures dropped to 5.5° C in about 15 inches of water and 6.1° C in about 3 feet of water in October. Also, in 1969, the highest mean monthly temperatures were recorded in July, values reaching as high as 23.3° C at 15 yards offshore. Values in the open lake were slightly lower in

comparison to those close to the shore. At 200 yards offshore recordings were 22.0° C in about 15 inches of water and 18.2° C in about 3 feet of water (Figure 5). An inverse thermal stratification was noted in October of 1968, with the warmer water being close to the bottom of the lake.

Oxygen concentrations did not follow the same pattern in the respective summers of 1968 and 1969. In 1968 a monthly mean of 9.0 ppm was recorded in May at 15 yards offshore in about 12 inches of water. Thereafter, values began to fall reaching a low of 4.0 ppm in October. In comparison, mean values in the open lake, at 200 yards offshore varied from 8.6 ppm in May to 5.3 ppm in October in about 15 inches of water and 8.5 ppm to 5.5 ppm at the same time, in about 3 feet of water. Values in 1969 were on the whole higher than those in 1968. At 15 yards offshore and in about 12 inches of water, the oxygen concentration varied from 12.3 ppm in May to 12.7 ppm in October (Figure 4). In the open lake, the oxygen concentration did not differ greatly from that close to the shore. At 200 yards offshore and at a depth of 15 inches, values varied from 11.0 ppm in May to 13.9 ppm in October and from 10.3 ppm to 13.9 ppm in the same period at a depth of 3 feet. At times of algal bloom, the oxygen concentration was very high; a value of 17.0 ppm was recorded during an algal bloom in 1969.

The field studies also included determinations of the

prevalence of cystacanths from January 1969 to December 1969. Figure 6 shows the prevalence of gammarids bearing the cystacanth stage of P. marilis at the location 15 yards offshore. There was an increase in the prevalence of cystacanths; the increase followed a pattern similar to that in temperature. The prevalence increased as from late April (5.5-8.5%) reaching a high of 29.3% in the first week of June. After the first week of June, the prevalence declined. Observed values were 25.6% to 24% at the end of June. At this time, many young gammarids were recruited into the gammarid population. The decrease in the prevalence of cystacanths in the gammarids continued through the fall months, reaching a low, with almost constant prevalence from October through December. Similar observations made in the open lake revealed the same pattern except that the prevalence was generally lower than that at 15 yards offshore.

Laboratory Observations

Natural infections with P. marilis in gammarids collected from under the ice. At 23° C, the prevalence of different larval stages of P. marilis in gammarids at time zero was minimal (Figure 7). The prevalence of early acanthellae increased to 6.3% after two weeks of incubation, then dropped to about 1.7% on the fourth week. The intermediate acanthellae were also most prevalent at two weeks, then dropped to 3.6% on the fourth week and to 0.4% on the sixth

Fig. 4. Seasonal variation in temperature and dissolved oxygen at Diver Bay, Cooking Lake, Alberta, 1968-1969.

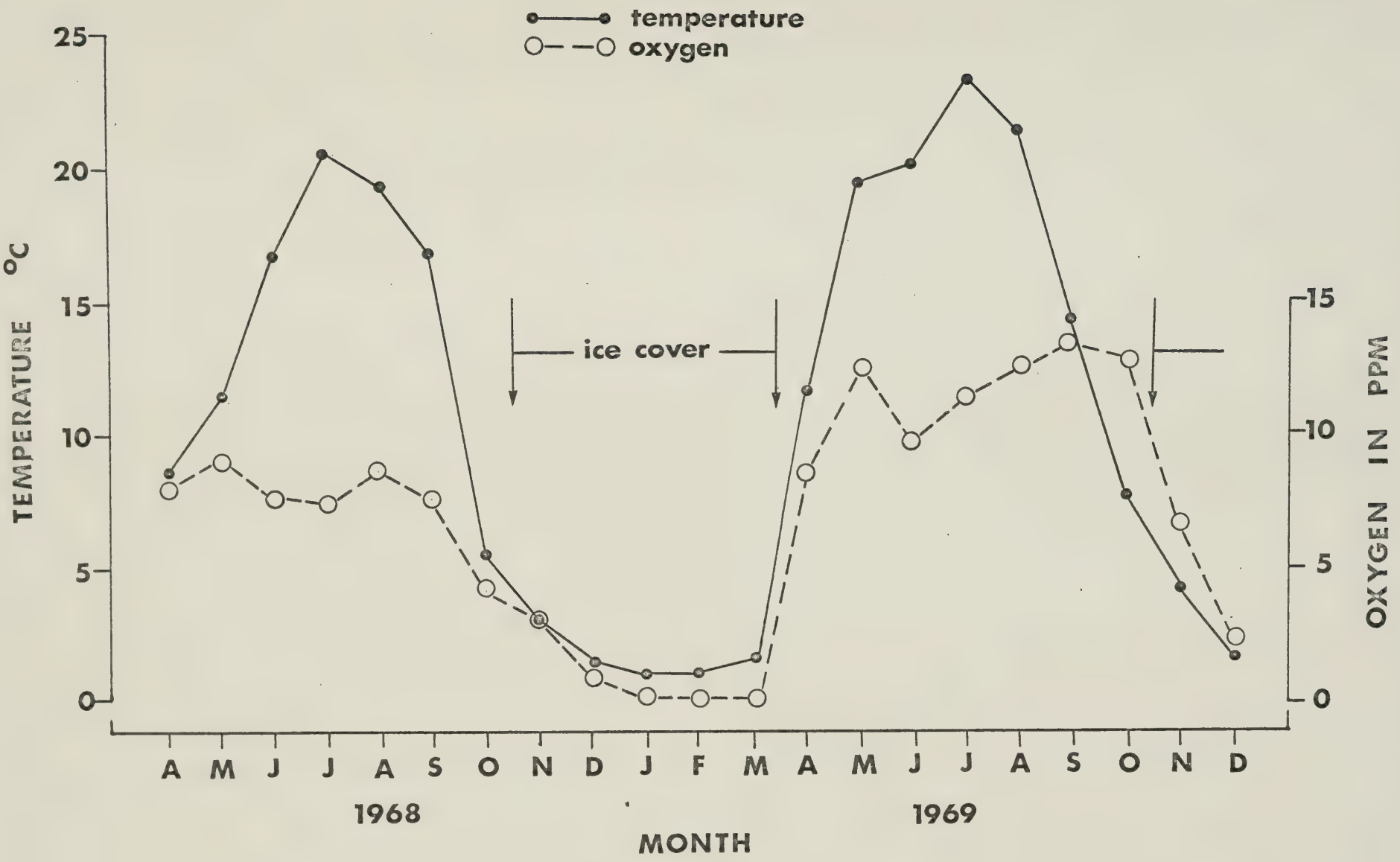

temperature
oxygen
ice cover
TEMPERATURE °C
OXYGEN IN PPM
25
20
15
10
5
0
15
10
5
0
A M J J A S O N D J F M A M J J A S O N D
1968
1969
MONTH

Fig. 5. Variation in temperatures at three stations at Diver Bay, Cooking Lake, Alberta, from breakup until freezeup, 1969.

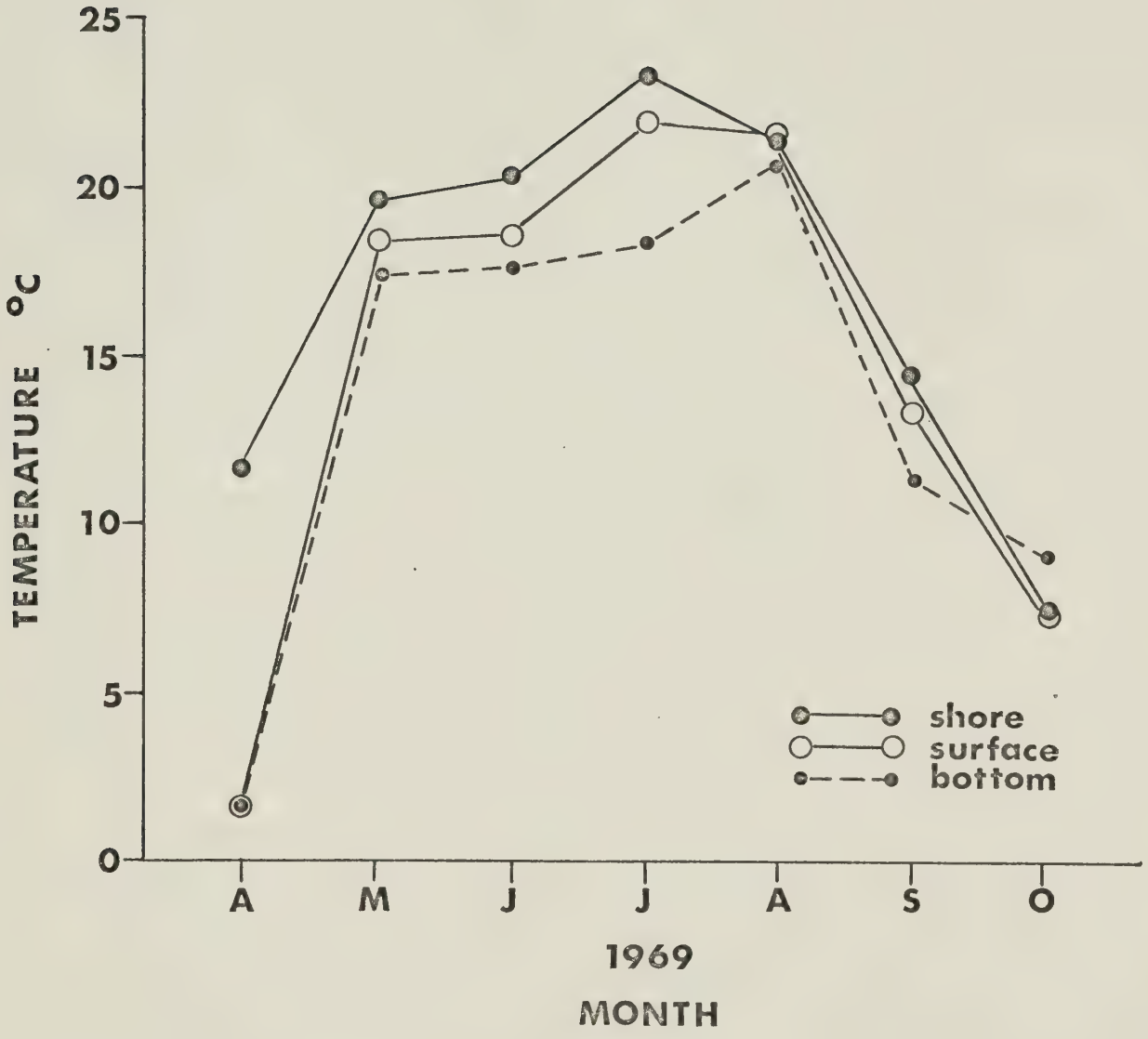
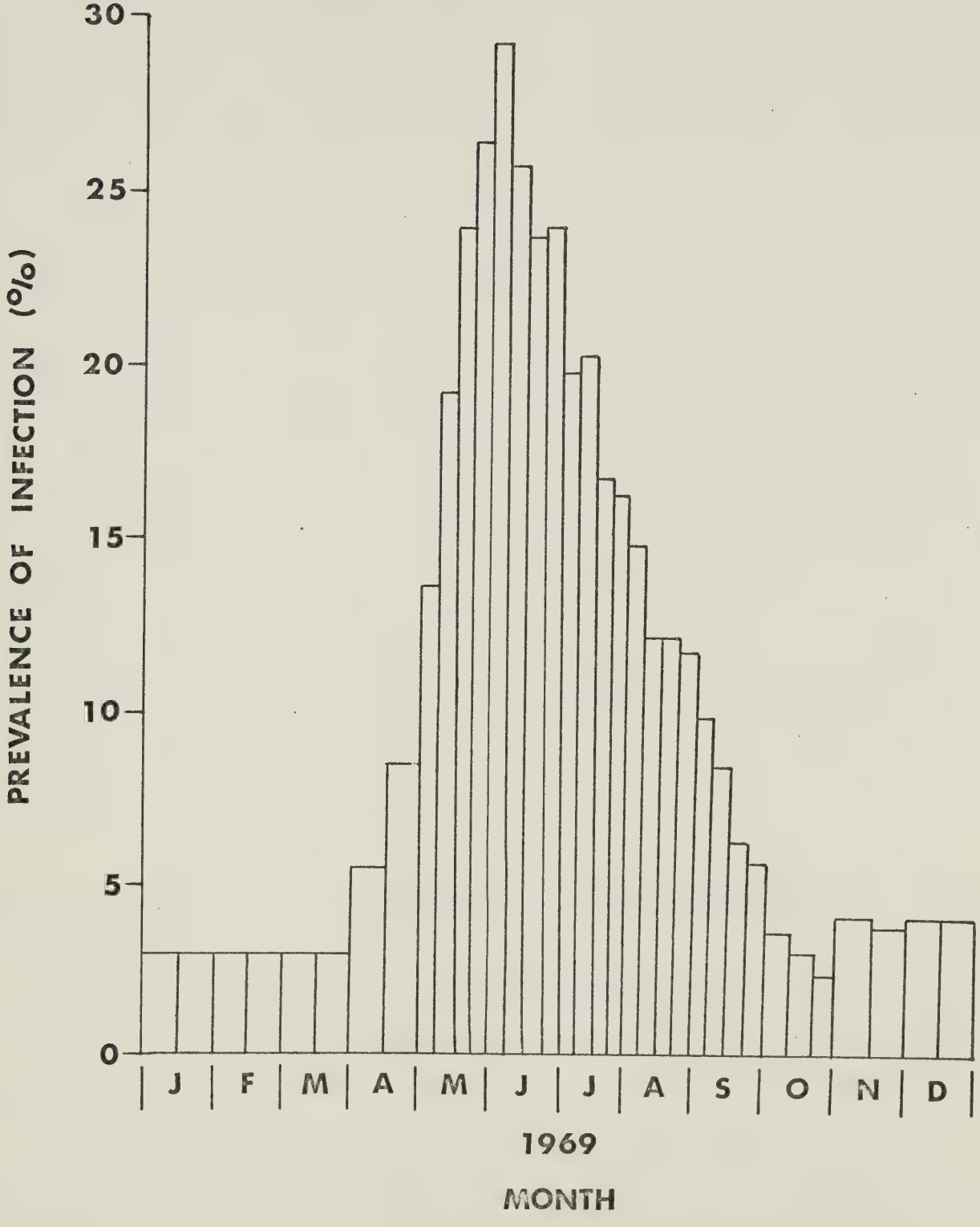

25
20
15
10
5
0
TEMPERATURE °C
A
M
J
J
A
S
O
1969
MONTH
shore
surface
bottom

Fig. 6. Prevalence of cystacanths of Polymorphus marilis in Gammarus lacustris in Diver Bay, Cooking Lake, Alberta, 1969. Each bar represents one collection.


PREVALENCE OF INFECTION (%)
30
25
20
15
10
5
0
J
F
M
A
M
J
J
A
S
O
N
D
1969
MONTH

week. The prevalence of advanced acanthellae increased on the fourth week to a peak, then dropped progressively on the sixth and eighth weeks. As the prevalence of the acanthella stages declined, that of fully developed cystacanths increased, reaching a maximum value of 17.7% on the eighth week.

At 15° C, the intervals between the peak prevalences reached by the different developmental stages were longer (Figure 7). For instance, the prevalence of the intermediate acanthella stage reached a peak on the fourth week as opposed to the second week at 23° C. The prevalence of fully developed cystacanths did not reach a peak until the twelfth week.

At 5° C there was no appreciable increase in prevalence of gammarids harbouring late developmental stages of P. marilis even after prolonged incubation at this temperature (Figure 7).

Development at Constant Temperatures in Laboratory-Infected Gammarids

The times required for the development of the larval stages of P. marilis at different constant temperatures are illustrated in Figure 8.

At 27° C, the gammarids die before the development of the larval stages can be completed. Twenty-five degrees Celsius was the highest temperature at which development was successfully completed. As demonstrated in Figure 8,

Fig. 7. Prevalence of each larval stage of Polymorphus marilis in naturally-infected gammarids collected under the ice, then maintained at a constant temperature in the laboratory. The time indicates weeks after collection. Stage 1 = early acanthella, Stage 2 = intermediate acanthella, Stage 3 = advanced acanthella, and Stage 4 = cystacanth.

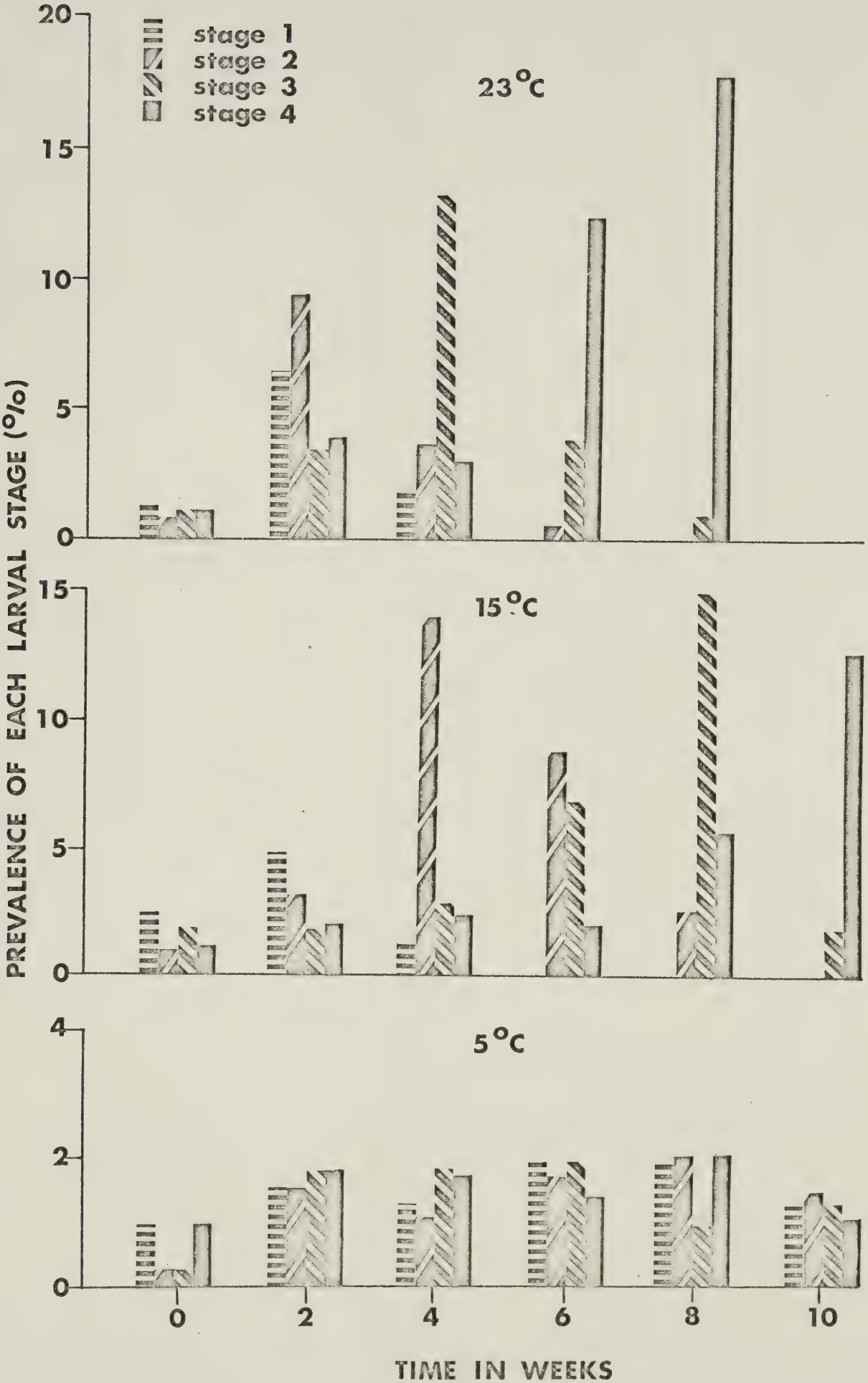
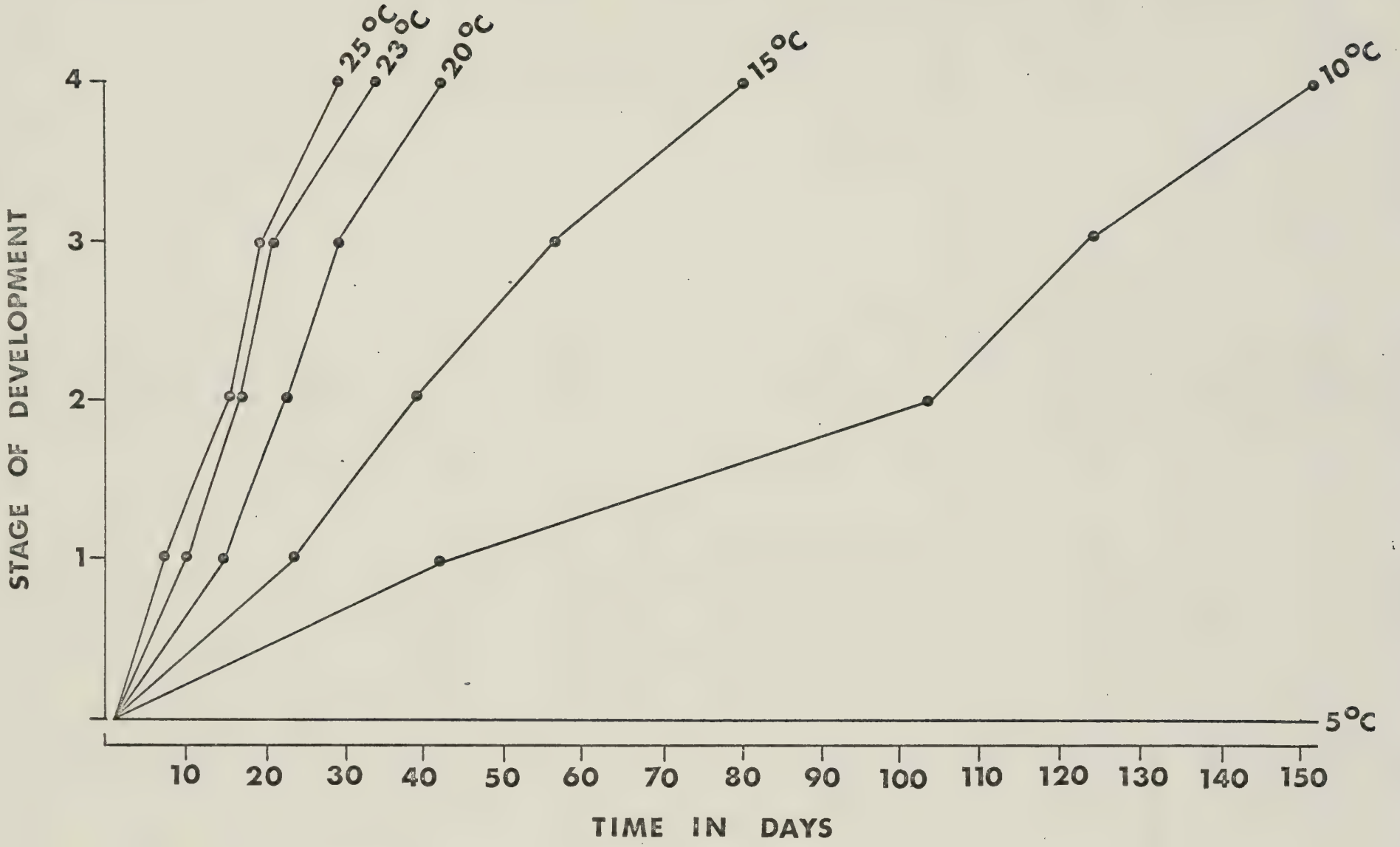

stage 1
stage 2
stage 3
stage 4
23°C
15°C
5°C
PREVALENCE OF EACH LARVAL STAGE (%)
TIME IN WEEKS
0
2
4
6
8
10
0
5
10
15
20
0
2
4

Fig. 8. Time of development of Polymorphus marilis in Gammarus lacustris maintained at different constant temperatures. Time = time taken for 50% of the larvae to reach the specified stage. Stage 1 = early acanthella, Stage 2 = intermediate acanthella, Stage 3 = advanced acanthella, and Stage 4 = cystacanth.


25°C
23°C
20°C
15°C
10°C
5°C
4
3
2
1
STAGE OF DEVELOPMENT
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
TIME IN DAYS

development was rapid, 50% reaching the cystacanth stage (stage 4) in 29.4 days. Successively lower temperatures produced increases in the time of development. At 23° C 50% reached the cystacanth stage in 34.1 days, an increase of approximately 16% with a drop in temperature of just 2° C. At 20° C developmental time increased to 42.2 days, an increase of about 44% and 29% from those incubated at 25° C and 23° C respectively. At 15° C the time taken to reach the cystacanth stage was 80.9 days--more than 3 times the time required at 25° C. At 10° C the impact of temperature was even greater, the time required (151.7 days) to reach 50% of cystacanth stage being about five times that required at 25° C.

To determine the relationship between temperature and rate of development, the logarithm of the reciprocal of the time required for 50% of the larvae to reach a particular stage of development (1/time of development, is the conventional way of expressing the rate of development) was plotted against temperature. The data approximate a straight line (plotted in Figure 9 by the method of least squares). A regression analysis of the data showed a highly significant linear regression of rate of development on temperature ($F = 137.8$; $p<0.001$), although the analysis also revealed a significant quadratic trend on temperature ($F = 244.7$; $p<0.001$), with the linear regression accounting for 98.6% of the variability. As a first approximation, therefore, the

relationship between log rate of development and temperature is here considered linear.

The developmental times and rates of development of the other stages at the different constant temperatures show patterns similar to that of the cystacanth stage (Figure 9). In all cases, the linear regression of log rate of development on temperature was highly significant and accounted for 90% or more of the variability in the data.

An added effect of development at low temperature was an increase in size (volume) of the cystacanths (Table 2). Cystacanths recovered from infection at low temperature (10° and 15° C) were larger than those at 20° and 23° C, although cystacanths recovered from infections at 25° C were significantly larger than those at 20° and 23° C.

Development at Fluctuating Temperatures

The acanthocephalan larvae that developed in alternate 48-hour periods at 15° C and at 25° C, showed a pattern similar to that of larvae kept at a constant temperature equal to the mean, 20° C (Table 3).

When the temperature were alternated between 5° C and 23° C, developmental times corresponded to the length of time spent at 23° C (Table 4), suggesting that all the development occurred at 23° C and that, as in previous controls, no development occurred at 5° C.

When infected gammarids were transferred to 23° C after 10-23 days at 5° C, the larval stages of *P. marilis* resumed

Fig. 9. Log of speed of development versus temperature for each stage of development of Polymorphus marilis in Gammarus lacustris. Stage 1 = early acanthella, Stage 2 = intermediate acanthella, Stage 3 = advanced acanthella, and Stage 4 = cystacanth.

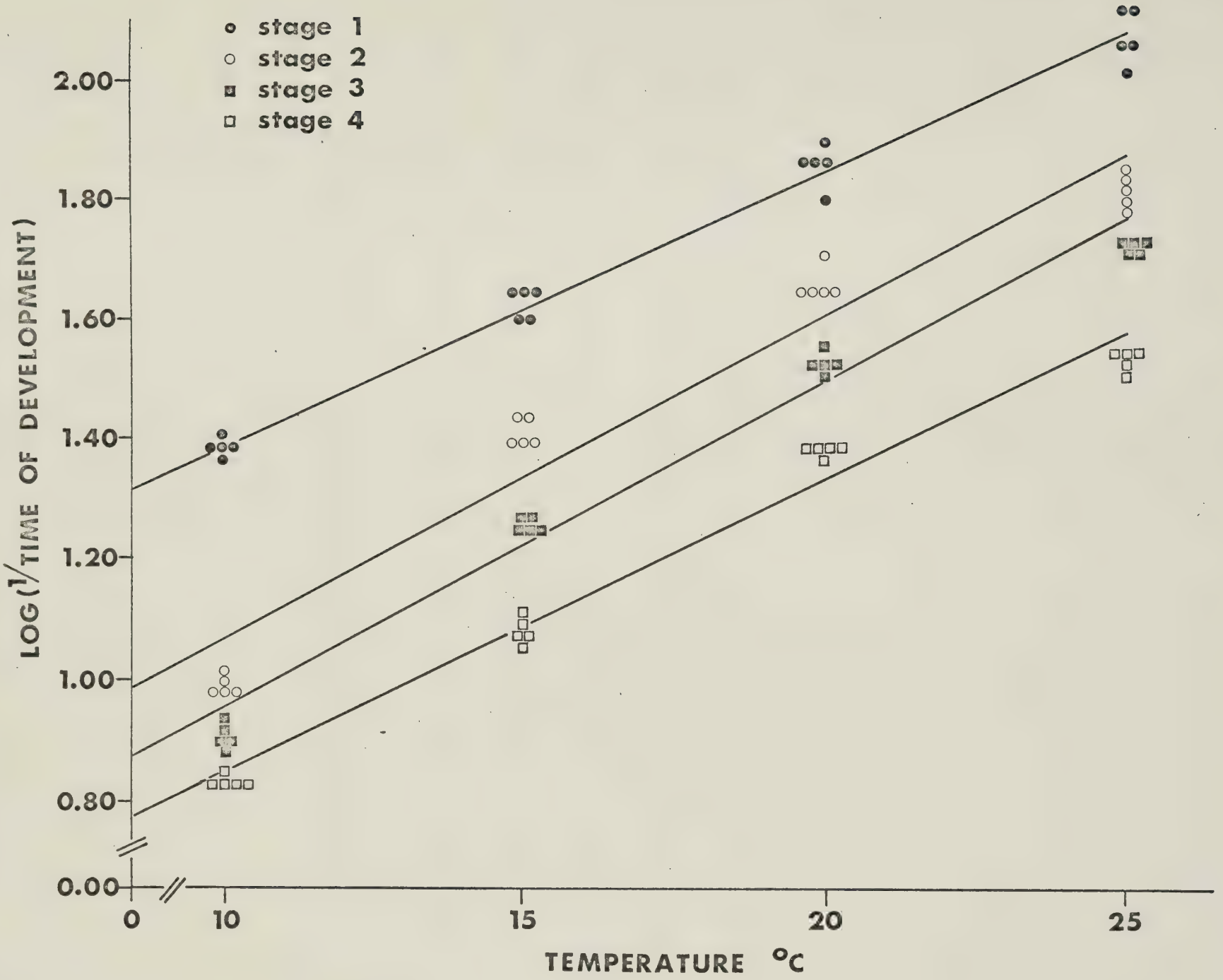

stage 1
stage 2
stage 3
stage 4
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.00
LOG (1/TIME OF DEVELOPMENT)
0
10
15
20
25
TEMPERATURE °C

Table 2. Effect of temperature on size (volume) of cystacanths.

Temperature (° C)	10	15	20	23	25
Mean volume (mm^3)[a]	1.27	1.14	0.95	0.92	1.03
Range of volumes	1.00-1.48	0.96-1.55	0.72-1.13	0.76-1.07	0.87-1.51

[a]Measurements of 30 of the cystacanths recovered from cultures at each of the constant temperatures were taken.

Table 3. Effect of alternating temperatures on the duration of the stages of development.

Stage	T_{50} (Days of Incubation) at temperature of		
	20	15[a]-25	25[a]-15
1	14.4	14.0	14.5
2	22.4	20.3	20.9
3	29.9	30.0	31.3
4	42.2	42.5	44.0

[a]Temperature of first exposure.

Table 4. Effect of alternating temperatures (one of which is below the threshold of development) on the duration of the stages of development.

Stage	Time at 23 ° - 5° C: Total number of days at 23° - 5° C	Time at 23 ° - 5° C: Days to 50% of stage at 23° C	Time at 23 ° - 5° C: Days at 5° C	Constant temperature (23° C)
1	21.0	11.0	10.0	10.0
2	33.3	17.3	16.0	16.8
3	44.5	22.5	22.0	21.1
4	68.8	34.8	34.0	34.1

and continued development at the normal rate (Table 5a). Fifty per cent of the larvae reached the cystacanth stage after 34.1 days at 23° C, the same time as in the control gammarids incubated at the constant temperature of 23° C. Stages of P. marilis that were incubated at 23° C until the early acanthella, intermediate or advanced acanthella were reached, then transferred to 5° C for as long as 210 days did not develop further (Table 5b).

Effects of Partial Development at 10° C

The development of larval acanthocephalans in gammarids incubated at 10° C for 28 or 40 days, then transferred to 23° C, was markedly different from their development at the control temperature of 23° C. As observed earlier, it took 151.7 days at 10° and 34.1 days at 23° to reach 50% of the cystacanth stage. Therefore, development for one day at 10° C would be equal to the development for 0.22 of a day at 23° C. Consequently, it was expected that cultures transferred from 10° C to 23° C after 28 days would have completed the equivalent of 6.2 days at 23°, and would require an additional 27.9 days to reach the 50% cystacanth level. Similarly, development in cultures transferred from 10° C to 23° C after 40 days would have completed the equivalent of 8.8 days at 23°, and would require an additional 25.3 days at 23° C. Actually, it took 47.7 days for those transferred from 10° C to 23° C after 28 days, approximately 71 % more time than expected, and 18.2 days for those transferred

Table 5. The effects on development when cultures are transferred from one temperature to another after a period of incubation at one temperature.

a. Cultures transferred from 5° C to 23° C

Time at 5° C before transfer to 23° C (days)	Stage (50% of stage)	Time at control temperature (23° C) (days)	Time at 23° C after transfer from 5 C (days)	Stage (50% of stage)
10	no development	10	10	1
18	no development	16.8	16.8	2
23	no development	21.1	21.1	3

b. Cultures transferred from 23° C to 5° C

Time at 23° C before transfer to 5° C (days)	Stage (50% of stage)	Time at control temperature (5° C) (days)	Time at 5° C after transfer to 23° C (days)	Stage (50% of stage)
10	1	210	210	no development
16.8	2	210	210	no development
21.1	3	210	210	no development

from 10° C to 23° C after 40 days, approximately 27% less time than was expected (Table 6). The same pattern of development prevailed for each of the acanthella stages. The elapsed time between stages was roughly two-thirds that for those in gammarids maintained at 23° C throughout their development and roughly one-third that for those in gammarids incubated at 10° C for 28 days (Table 6). The results suggest a phenomenon more complicated than dormancy.

Development at Reduced Oxygen Concentration

The larval stages of P. marilis were found to develop normally at dissolved oxygen concentration of 1.4 ppm and 4.0 ppm. The time of development for each stage was comparable to that at the control conditions (23° C and 7.3 ppm oxygen concentration (Table 7)). It was impossible to test development in a completely anoxic environment in the laboratory, since gammarids died in 12-24 hours, depending on temperature, in completely anoxic conditions.

Table 6. Effect on development at 23° C after transfer from 10° C at various incubation times.

	Time in days to 50% of stage at control temperature		Time in days to 50% of stage after transfer to 23° C	
Stage	10° C	23° C	A[a]	B[b]
1	42.0	10.0	28+10.0	
2	103.3	16.8(6.8)[c]	28+17.7(7.7)	40+7.1
3	122.1	21.1(4.3)	28+29.3(11.6)	40+11.1(4.0)
4	151.7	34.1(13.0)	28+47.7(18.4)	40+18.2(7.1)

[a] 28 days at 10° C + stated days at 23° C.

[b] 40 days at 10° C + stated days at 23° C.

[c] Values in parenthesis are times elapsed from last stage.

Table 7. Effect on development at reduced oxygen concentration in 23° C.

Culture	Oxygen content (ppm)	Time (days) to 50% of developmental stage 1	2	3	4
A (control)	7.3	10	16.8	21.1	34.1
B	4.0	10	16.8	21.1	34.1
C	1.4	10	16.8	21.1	34.1

DISCUSSION

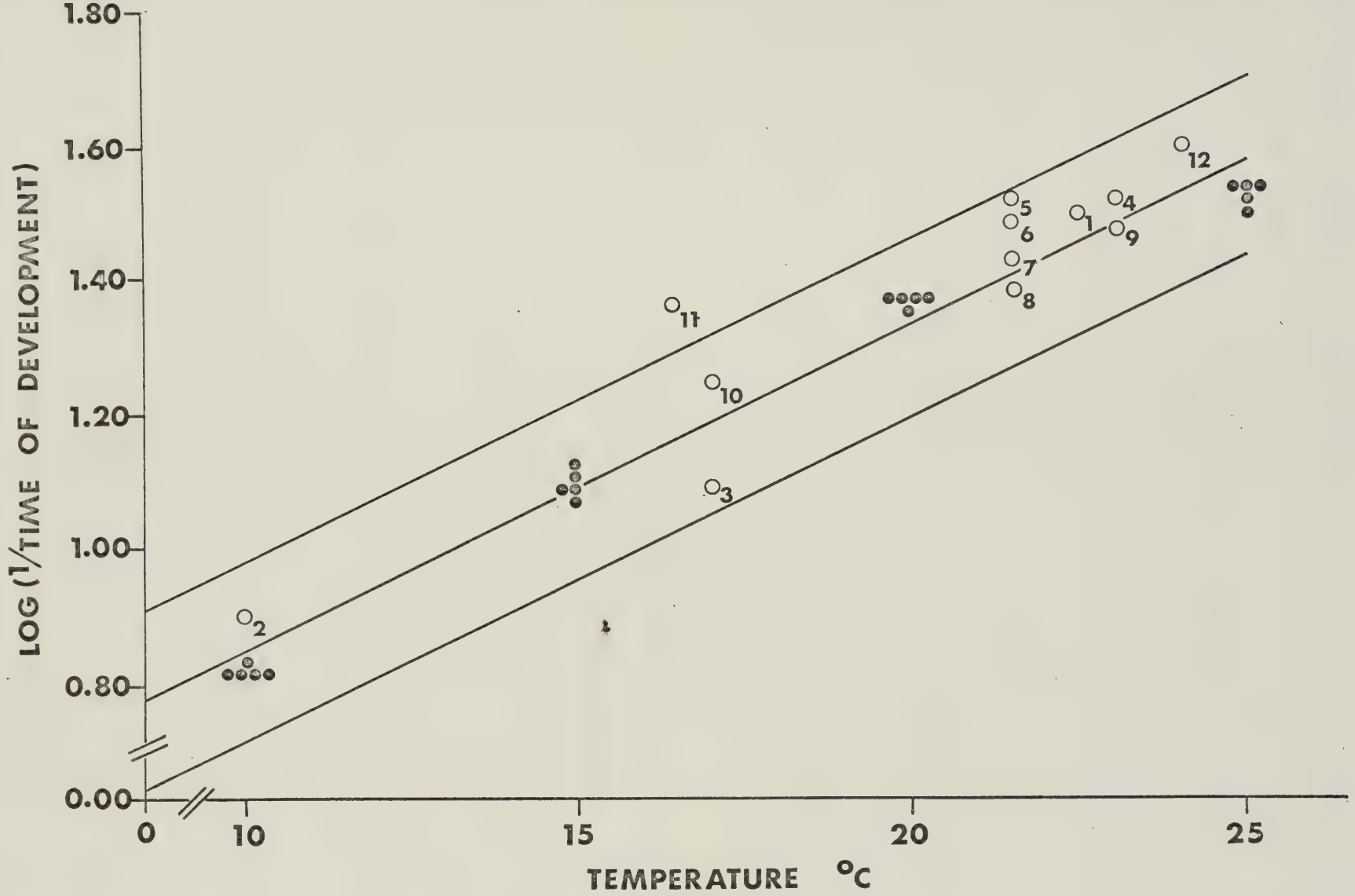
The results of the present study indicate that temperature is an important environmental factor influencing the development of the larval stages of *P. marilis* in *G. lacustris*, but that variations in the dissolved oxygen concentration have no effect on their development.

Polymorphus marilis can successfully complete its development in gammarids through a fairly wide range of temperatures. The threshold temperature of development is above 5° C (at which no development takes place), but below 10° C. Normal development occurs at temperatures as high as 25° C, just below the thermal lethal temperature for the local populations of *G. lacustris*. Within this favorable temperature range, the logarithm of the speed of development is essentially linearly related to temperature.

No previous study has attempted to determine the threshold of development for an acanthocephalan, but several studies have reported times required by various palaeacanthocephalans to complete their morphological development at various temperatures. When these times are converted to speed of development and the logarithms plotted against temperature, the points fall close to the line calculated from my data on *P. marilis* (Figure 10). All but one fell within two standard errors of the line calculated for *P. marilis*. The exception was one of two values determined by Romanovsky (1964).

Fig. 10. Log of speed of development versus temperature for the cystacanths of various Palaeacanthocephala, compared with that for Polymorphus marilis. Solid dots show values for P. marilis, the lines show the regression calculated for those values (middle line) ± two standard errors (outer lines). Open circles indicate values calculated for other Palaeacanthocephala from data in the following papers:

1. Leptorhynchoides thecatus in Hyalella azteca (Degiusti, 1949)
2. Polymorphus minutus in Gammarus pulex (Butterworth, 1969)
3. Echinorhynchus truttae in Gammarus pulex (Awachie, 1966)
4. Echinorhynchus lageniformis in Corophium spinicorne (Olson and Pratt, 1971)
5. Coryonosoma constrictum in Gammarus lacustris (Podesta and Holmes, 1970a)
6. Polymorphus contortus in Gammarus lacustris (Podesta and Holmes, 1970a)
7. Polymorphus contortus in Hyalella azteca (Podesta and Holmes, 1970a)
8. Polymorphus trochus in Gammarus lacustris (Podesta and Holmes, 1970a)
9. Polymorphis marilis in Gammarus lacustris (Denny, 1967)
10. Polymorphus minutus in Gammarus pulex (Hynes and Nicholas, 1957)
11. Polymorphus minutus (Romanovsky, 1964)
12. Polymorphus minutus (Romanovsky, 1964)


1.80
1.60
1.40
1.20
1.00
0.80
0.00
LOG (1/TIME OF DEVELOPMENT)
0
10
15
20
25
TEMPERATURE °C
1
2
3
4
5
6
7
8
9
10
11
12

Romanovsky's (1964) value of 44 days at 16.4° C was considerably more than two standard error above the line, although his value of 25 days at 24° C fell within that range. These results suggest that palaeacanthocephalans appear to show similar relationships between speed of development and temperature.

Although most laboratory studies dealing with the relationship between temperature and speed of development are conducted at constant temperatures, temperatures under natural conditions fluctuate diurnally and seasonally. Consequently, in this study an attempt has been made to observe the development of P. marilis at fluctuating temperatures.

Andrewartha and Birch (1954:162) have pointed out that the rate of development with short-term fluctuations in temperatures within the favorable range may safely be considered to be the equivalent of the rate at a constant temperature of the same mean. My results with temperatures alternating between 15° and 25° C agree with this generalization.

When the fluctuations involve temperatures outside the favorable range (below the threshold of development) the situation is more complex. When the gammarids were incubated alternately at 5° and 23° C, the time of development was shorter than that at the control temperature (15° C, compare Table 3 and Figure 7), but equivalent to the time spent at 23° C. All of the development must have occurred at 23° C,

at a rate more than double that at 15° C. This lack of development at 5° C has been attributed to dormancy. A similar situation was observed when cultures were transferred from 23° C to 5° C after partial development at 23° C, suggesting that any of the acanthella stages of P. marilis can enter into a state of dormancy when the environmental temperature is unfavorable.

Furthermore, experiments in which the development of P. marilis was followed in naturally-infected gammarids collected from under the ice in winter (Denny, 1967; my first experiment) showed that dormant early stages were present in overwintering gammarids.

Although dormancy has received considerable attention and the subject has been treated in detail by Lees (1955), there is little known about this phenomenon in acanthocephalans. There are two main types of dormancy. The simpler is termed quiescence and is characterized by a cessation of activity during exposure to unfavorable conditions. This is simply an immediate and direct response to an unfavorable environment. The dormancy shown by P. marilis at 5° C in the laboratory or under the ice in nature is of this type.

There is another more complex physiological dormant condition, very prevalent in insects, known as diapause. The onset of diapause, in some cases, may occur well in advance of drastic environmental conditions. Diapause occurring irrespective of environmental conditions is termed obligatory diapause. Diapause in which the onset is controlled by

environmental "signals" such as temperature, length of day, or rate of change of day is termed facultative diapause.

It is suggested that facultative diapause was observed in the present study in the experiment in which infected gammarids were incubated for various times at 10° C, then were returned to 23° C. The retardation in development in larvae transferred after 28 days is attributed to the lingering effect of an incomplete facultative diapause. Retardation in development of this kind has been demonstrated in insects when the diapause has not been terminated (Lees, 1955). For those transferred after 40 days, development was accelerated, suggesting that the diapause had been completed by that time and that development was not impeded by its effects. Acceleration of this type has also been shown in copepods (Elgmork, 1959). This pattern of events, with a major difference in the effects of incubation at 5° and 10° C, is in agreement with Andrewartha and Birch's (1954) suggestion that the onset of diapause influenced by temperature occurs only at appropriate temperature and that diapause termination and the resumption of normal and even accelerated growth depends on the length of incubation at the appropriate temperature. Further observations on the phenomenon of dormancy in P. marilis are necessary to obtain enough information to draw definite conclusions. The physiology of diapause-like dormancy may be a good place to start.

Field surveys and the results obtained in the laboratory

suggest that the life cycle of P. marilis in nature is bivoltine and operates in synchrony with the seasons. Graham (1966) found that there are two peaks of infections of adult worms in lesser scaups--the first in early July and the second in about mid-August.

Scaups from their wintering ground arrive at the lake in late spring and stay around the lakes for a period of about six months. In late May and early June the scaups become infected with the increasing number of cystacanths which have developed from the dormant overwintering early larval stages. By July, embryonated eggs or gravid female worms are passed out and infect more gammarids. The temperature of the lake being high at this time, development of the larval acanthocephalans proceeds rapidly and cystacanths are available to infect scaups in early August. By the end of August or early September, young gammarids are infected from eggs passed by gravid adult female worms. Development of the larval acanthocephalans in gammarids infected in the fall when the temperatures begin to drop considerably is slow; by the onset of winter development has rarely proceeded past the early acanthella stage. This becomes dormant when the temperature drops below the threshold of development.

Denny (1967) found a marked decrease in the prevalence of cystacanths in gammarids over winter, and suggested that gammarids infected with the large larval parasites suffered selective mortality. Therefore, it would be to the parasite's

advantage not to develop to the cystacanth stage by the onset of winter.

Assuming that the diapause is a facultative phenomenon, responding to temperatures around 10° C (or possibly to the declining temperature characteristic of Cooking Lake in late August and September), the retardation in development after a relatively short period of diapause and subsequent return to more favorable temperatures would be an adaptation to guard against reaching the cystacanth stage during short-lived "Indian Summer" conditions. Such conditions occur in central Alberta.

Although the possibility of accelerated development in the spring was not investigated, the accelerated development following a long period of diapause suggests that such an acceleration may occur under spring conditions. Further study of this phenomenon is definitely indicated.

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APPENDIX I

Table 1. Water temperatures and dissolved oxygen concentrations recorded in Diver Bay, Cooking Lake at selected distances from the shore and at selected water depths. Recordings apply to the period April to December 1968.

Date	Time	Water temperatures: Distance from shore / Water depths	15 yds. 12 in.	200 yds. 15 in.	3 ft.	Dissolved oxygen concentrations: Distance from shore / Water depths	15 yds. 12 in.	200 yds. 15 in.	3 ft.
April									
26	1300		8.5	8.0	9.0		8.0	8.0	5.4
30	1300		8.5	9.5	7.5		8.0	8.5	7.5
May									
9	1100		8.0	8.2	8.0		8.3	8.3	8.0
18	1000		12.4	11.6	10.8		10.4	9.0	8.6
30	1100		13.0	14.0	13.2		8.9	8.5	8.5
	1200		14.0	16.0	15.0		8.5	8.6	8.9
June									
6	1000		16.0	15.0	13.0		9.4	8.5	8.9
	1100		16.0	15.0	13.0		9.4	8.5	8.9
	1200		16.5	15.0	14.0		8.9	9.1	7.8
	1300		17.0	16.0	15.0		9.1	9.9	8.2
14	1200		14.5	13.0	14.0		6.6	7.6	6.9
	1300		16.0	15.0	13.0		6.4	7.8	6.7
	1400		16.5	15.0	15.5		8.8	9.0	8.8
	1500		16.3	15.2	15.5		8.8	9.0	8.8
30	0900		18.0	17.0	16.0		4.2	5.0	4.8
	1000		18.0	17.0	16.0		4.2	4.9	4.6
	1100		18.0	19.0	16.0		6.9	7.5	7.3
	1200		18.5	19.0	17.0		6.9	7.5	7.3
	1300		19.0	19.0	17.0		7.5	7.7	7.2
	1400		19.0	19.0	17.0		7.2	7.7	7.7

Table 1. Continued

Date	Time	Distance from shore / Water depths	Water temperatures: 15 yds. 12 in.	200 yds. 15 in.	200 yds. 3 ft.	Distance from shore / Water depths	Dissolved oxygen concentrations: 15 yds. 12 in.	200 yds. 15 in.	200 yds. 3 ft.
July									
4	1400		27.5	27.0	19.0		6.9	8.0	7.8
	1500		27.0	28.0	19.5		6.9	8.0	7.8
	1600		23.0	24.0	21.0		8.0	9.4	8.9
15	1000		15.0	16.0	16.0		5.6	6.4	5.6
	1100		16.5	16.0	16.0		8.0	7.0	6.4
22	1300		18.0	19.6	18.8		6.3	6.3	6.1
27	1030		18.6	18.0	16.2		8.6	10.8	8.0
	1130		21.5	20.0	17.0		9.0	10.5	8.5
	1230		22.5	21.0	18.0		9.1	10.5	8.5
	1330		22.2	21.0	17.0		10.0	11.8	9.4
	1430		22.2	22.0	19.0		10.0	11.8	9.4
	1530		22.0	21.0	19.5		10.2	12.1	9.4
	1630		21.9	22.0	19.0		-	9.6	-
	1730		21.2	21.0	18.0		-	-	-
Aug.									
3	1400		26.2	25.9	23.6		8.9	10.4	8.8
13	1540		16.7	15.0	15.0		9.0	9.8	9.0
18	1200		19.6	20.5	20.1		8.0	7.7	7.5
25	1400		14.0	14.0	13.3		8.6	8.8	7.6
Sept.									
14	1100		19.0	14.2	15.0		-	7.4	6.7
	1200		18.0	16.0	15.0		7.4	7.6	4.4
	1400		20.0	16.5	15.0		7.6	7.1	5.6
	1530		18.5	17.0	15.0		9.0	9.3	6.4
	1630		18.5	16.0	15.2		9.0	9.6	7.6
22	1300		14.6	14.0	13.3		6.9	8.2	7.6
29	1200		14.6	14.0	13.3		6.9	8.2	7.6
	1300		14.6	14.0	13.3		6.9	8.2	7.6

Table 1. Continued

Date	Time	Distance from shore / Water depths	Water temperatures: 15 yds. 12 in.	200 yds. 15 in.	200 yds. 3 ft.	Distance from shore / Water depths	Dissolved oxygen concentrations: 15 yds. 12 in.	200 yds. 15 in.	200 yds. 3 ft.
Oct.									
12	1400		11.9	11.6	10.5		5.4	6.8	6.4
19	1000		1.0	2.0	3.0		3.2	4.8	4.9
26	1500		3.0	3.0	5.0		3.5	4.5	5.2
				Under Ice					
Nov.									
6	1300		5.2				3.0		
15	1400		2.5				3.1		
28	1200		1.5				3.1		
Dec.									
10	1200		1.5				1.2		
19	1200		0.0				0.9		
29	1300		2.1				0.9		

APPENDIX II

Table 1. Water temperatures and dissolved oxygen concentrations recorded in Diver Bay, Cooking Lake at selected distances from the shore and at selected water depths. Recordings apply to the period late April to late October 1969.

Date	Time	Distance from shore / Water depths	Water temperatures (° C) 15 yds. 12 in.	200 yds. 15 in.	3 ft.	Distance from shore / Water depths	Dissolved oxygen (ppm) 15 yds. 12 in.	200 yds. 15 in.	3 ft.
April									
22	1300		11.4	1.5[a]	1.5[a]		8.5	1.8[a]	-
30	1400		11.8	1.5[a]	1.5[a]		8.5	1.8[a]	-
May									
10	1300		16.5	16.0	14.9		12.9	13.6	9.0
13	1430		23.9	23.0	23.0		13.7	12.4	9.8
22	1345		21.2	19.0	16.5		12.8	9.5	11.2
28	1215		16.5	15.2	14.9		10.0	8.5	11.2
June									
7	1830		21.0	21.5	20.5		14.1	12.1	10.5
16	1330		22.5	19.7	19.5		6.8	10.2	9.1
20	1200		19.0	17.0	17.0		8.8	10.3	8.5
30	1330		18.0	16.5	14.7		8.5	10.0	9.2
July									
1	1500		24.0	22.0	19.6		12.8	13.3	11.2
15	1355		26.0	23.0	17.0		12.1	13.1	11.9
25	1250		20.0	21.0	18.0		8.9	5.7	5.7
Aug.									
12	1450		21.0	21.6	22.4		11.4	10.1	8.6
21	1600		19.8	22.5	22.5		13.1	16.2	15.8
28	1310		22.4	21.2	21.5		12.1	12.9	10.6
31	1055		21.4	21.5	21.5		12.9	12.4	10.5

Table 1. Continued

Date	Time	Distance from shore / Water depths	Water temperatures (° C) 15 yds. 12 in.	200 yds. 15 in.	3 ft.	Distance from shore / Water depths	Dissolved oxygen (ppm) 15 yds. 12 in.	200 yds. 15 in.	3 ft.
Sept.									
10	1200		17.4	16.5	15.5		15.5	17.2	15.5
15	1200		15.5	14.2	11.2		11.2	11.4	11.6
22	1300		13.6	12.2	9.9		12.9	13.9	13.7
28	1300		11.5	10.3	8.5		13.2	14.7	13.2
Oct.									
4	1200		9.5	9.2	10.8		13.4	14.8	13.4
16	1400		5.8	4.5	7.2		12.0	13.0	12.4

[a]Temperatures and oxygen taken from under the ice.

APPENDIX III

Table 1. Prevalence of cystacanths of Polymorphus marilis in gammarids in Diver Bay, 1969 at 15 yards offshore and at 200 yards offshore.

Date		15 yards offshore		200 yards offshore	
		Gammarids examined/infected	Percent infection	Gammarids examined/infected	Percent infection
January	15	300(9)	3.0	300(5)	1.6
	31	300(9)	3.0	300(2)	0.6
February	15	270(8)	3.0	280(7)	2.5
	25	267(8)	3.0	267(7)	2.6
March	14	300(9)	3.0	300(5)	1.6
	25	296(9)	3.0	300(8)	2.6
April	15	305(17)	5.5	300(10)	3.3
	30	303(26)	8.5	300(14)	4.6
May	10	300(41)	13.6	300(25)	8.3
	13	301(58)	19.2	300(21)	7.0
	22	250(60)	24.0	250(18)	7.2
	28	212(56)	26.4	250(26)	10.4
June	7	368(108)	29.3	300(55)	18.3
	15	246(63)	25.6	300(39)	13.0
	20	268(64)	23.8	290(38)	13.1
	28	266(64)	24.0	301(42)	13.9
July	1	301(60)	19.8	301(36)	11.9
	15	303(61)	20.2	300(35)	11.6
	25	300(50)	16.6	300(35)	11.6
	31	296(48)	16.2	300(30)	10.0
August	12	311(46)	14.7	305(31)	10.1
	21	302(37)	12.2	310(28)	9.0
	28	302(37)	12.2	300(25)	8.3
	31	303(36)	11.8	300(19)	6.3

Table 1. Continued

Date		15 yards offshore		200 yards offshore	
		Gammarids examined/infected	Percent infection	Gammarids examined/infected	Percent infection
September	10	304(30)	9.8	300(23)	7.6
	15	306(26)	8.4	300(18)	6.0
	22	302(19)	6.2	300(16)	5.3
	28	300(17)	5.6	300(16)	5.3
October	4	301(11)	3.6	300(11)	3.6
	16	300(9)	3.0	270(8)	2.9
	27	290(7)	2.4	275(7)	2.5
November	14	270(11)	4.0	300(13)	4.3
	21	262(10)	3.8	300(10)	3.3
December	15	275(11)	4.0	300(15)	5.0
	31	270(11)	4.0	300(12)	4.0

APPENDIX IV

Table 1. Summarized data on the duration of larval development of Polymorphus marilis at different constant temperatures (second experiment).

Replicates	Days Post Infection	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae
				10° C					
	42	50(36)	72.0	110	100.0	-	-	-	-
	102	50(33)	66.0	47	21.2	29.7	-	-	49.0 G-S
	105	40(27)	67.5	78	-	96.7	-	-	3.2 G-S
A	121	40(32)	80.0	110	9.0	37.2	53.6	-	-
	125	40(24)	60.0	209	-	15.1	84.8	-	-
	148	50(41)	82.0	367	-	4.0	32.4	9.5	53.9 A-C
	155	63(47)	74.6	161	-	-	-	88.4	11.5 A-C
	42	50(41)	82.0	268	100.0	-	-	-	-
	102	50(28)	56.0	208	28.3	14.4	-	-	57.2 G-S
	106	50(39)	78.0	174	1.7	75.2	-	-	22.9 G-S
B	121	50(46)	92.0	259	-	75.2	24.7	-	-
	125	35(35)	100.0	158	-	10.1	89.9	-	-
	150	40(31)	77.5	150	-	-	59.3	21.3	19.3 A-C
	157	38(26)	68.4	235	-	-	8.0	88.9	2.9 A-C
	41	50(35)	70.0	123	100.0	-	-	-	-
	102	40(39)	97.5	308	20.7	16.2	-	-	62.9 G-S
	109	40(32)	80.0	173	-	89.0	-	-	10.9 G-S
C	128	40(26)	65.0	143	-	70.6	25.8	-	3.4 G-S
	131	40(35)	87.5	134	-	11.9	88.0	-	-
	150	50(48)	96.0	183	-	-	30.6	19.1	50.2 A-C
	157	57(42)	73.6	235	-	1.2	2.5	93.6	2.5 A-C

Table 1. Continued

Replicates	Days Post Infection	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae
				10° C					
	42	50(50)	100.0	233	100.0	-	-	-	
	100	50(31)	62.0	89	26.9	37.0	-	-	35.9 G-S
	103	40(33)	82.5	109	7.3	77.9	-	-	14.6 G-S
D	115	40(40)	100.0	82	-	53.6	46.3	-	-
	121	40(29)	72.5	158	-	12.0	87.9	-	-
	150	50(43)	86.0	115	-	-	4.3	22.6	73.0 A-C
	153	31(31)	100.0	183	-	4.1	10.5	78.8	-
	43	50(42)	84.0	203	100.0	-	-	-	-
	102	50(29)	58.0	132	15.2	17.5	-	-	67.2 G-S
	105	50(38)	76.0	104	17.3	59.6	-	-	23.0 G-S
E	123	35(31)	88.5	221	-	36.6	46.6	-	6.7 G-S
	126	35(33)	94.2	181	-	9.3	90.6	-	-
	148	30(27)	90.0	217	-	-	11.0	20.7	68.2 A-C
	150	29(29)	100.0	101	-	-	6.9	76.2	16.8 A-C
				15° C					
	25	60(45)	75.0	87	100.0	-	-	-	-
	40	50(40)	90.0	280	43.5	38.2	-	-	18.3 G-S
	44	50(42)	84.0	146	-	80.9	-	-	19.1 G-S
A	54	30(24)	80.0	57	-	56.1	43.6	-	-
	59	30(30)	100.0	194	-	17.3	82.7	-	-
	78	40(25)	62.5	160	-	27.5	22.5	50.0	-
	84	30(30)	100.0	111	-	-	5.4	94.6	-

Table 1. Continued

Replicates	Days post infection	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae
				15° C					
	25	40(30)	75.0	208	100.0	-	-	-	-
	35	40(20)	50.0	182	33.6	34.6	-	-	31.8 G-S
	39	40(28)	70.0	178	-	64.0	-	-	36.0 G-S
B	54	50(35)	70.0	286	-	50.3	45.5	-	4.2 G-S
	62	50(31)	62.0	126	-	10.0	90.0	-	-
	84	30(27)	90.0	118	-	27.1	28.0	44.9	-
	96	37(30)	83.8	111	-	-	5.0	95.0	-
	23	35(35)	100.0	221	100.0	-	-	-	-
	35	40(31)	77.5	227	51.9	20.5	-	-	27.5 G-S
	40	40(39)	97.5	136	-	94.8	-	-	5.1 G-S
C	54	40(31)	77.5	165	-	66.0	33.9	-	-
	62	40(25)	62.5	104	-	17.8	82.1	-	-
	78	50(36)	72.0	173	-	-	25.4	31.7	42.7 A-C
	89	43(41)	95.3	212	-	3.3	13.2	80.6	-
	23	50(25)	50.0	88	98.8	-	-	-	0.1 A
	40	35(31)	92.5	167	20.3	48.5	-	-	31.1 G-S
	42	40(32)	80.0	308	15.5	69.4	-	-	14.9 G-S
D	54	40(28)	70.0	156	-	68.7	29.1	-	2.0 G-S
	60	40(31)	77.5	155	-	12.9	87.0	-	-
	78	35(33)	94.2	112	-	-	31.2	39.6	29.0 A-C
	89	30(30)	100.0	104	-	20.5	5.1	74.3	-

Table 1. Continued

Replicates	Days post infection	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae
				15° C					
E	23	50(41)	82.0	226	100.0	-	-	-	-
	40	50(37)	74.0	303	58.7	30.4	-	-	11.2 G-S
	42	35(27)	77.1	148	12.3	67.7	-	-	20.0 G-S
	54	35(28)	80.0	192	-	55.9	30.8	-	13.3 G-S
	60	30(30)	100.0	211	-	15.8	78.7	-	6.1 G-S
	78	30(25)	83.3	104	-	19.7	20.7	31.2	23.7 A-C
	89	39(38)	97.4	234	-	-	6.0	71.3	22.5 A-C
				20° C					
A	13	50(45)	90.0	218	100.0	-	-	-	-
	22	50(32)	64.0	237	13.2	14.9	-	-	71.7 G-S
	24	50(39)	78.0	205	3.3	86.7	-	-	9.9 G-S
	29	40(31)	77.5	130	-	77.6	22.3	-	-
	31	35(22)	62.8	135	-	15.5	80.7	-	3.7 G-S
	40	35(30)	85.7	117	-	-	50.4	11.1	38.4 A-C
	43	23(21)	91.3	129	-	-	7.2	7.37	18.9 A-C
B	14	50(41)	82.0	151	100.0	-	-	-	-
	24	50(29)	58.0	136	5.4	83.1	-	-	11.4 G-S
	32	50(49)	98.0	421	-	6.6	91.4	-	1.9 G-S
	40	40(38)	95.0	139	-	-	22.6	9.3	67.9 A-C
	43	53(32)	60.3	126	-	-	-	84.4	15.5 A-C

Table 1. Continued

Replicates	Days post infection	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae
				20° C					
	14	40(31)	77.5	172	100.0	-	-	-	-
	22	40(36)	90.0	314	33.7	12.4	-	-	53.8 G-S
	25	40(29)	72.5	135	-	97.0	-	-	2.9 G-S
C	29	40(31)	77.5	161	-	72.2	27.7	-	-
	33	50(43)	86.0	198	-	5.3	94.8	-	-
	40	30(28)	93.3	201	-	-	12.4	9.5	77.9 A-C
	43	36(34)	94.4	120	-	-	8.3	83.2	8.3 A-C
	14	40(38)	95.0	190	100.0	-	-	-	-
	22	40(40)	100.0	341	13.1	12.6	-	-	74.1 G-S
	25	40(29)	72.5	123	3.0	87.5	-	-	9.4 G-S
D	29	40(33)	82.5	157	-	85.3	14.6	-	-
	31	40(30)	75.0	110	-	17.2	82.7	-	-
	40	40(31)	77.5	156	-	-	25.6	10.2	64.1 A-C
	43	31(31)	100.0	183	-	-	10.3	78.1	11.4 A-C
	17	50(41)	82.0	263	100.0	-	-	-	-
	22	40(40)	100.0	263	16.6	18.0	-	-	65.3 G-S
	24	40(31)	77.5	198	15.1	78.7	-	-	6.0 G-S
E	29	30(28)	93.3	220	5.4	78.1	12.7	-	-
	32	30(29)	96.6	156	-	10.8	81.2	-	7.9 G-S
	40	30(26)	86.6	195	-	4.9	9.0	11.4	74.5 A-C
	47	32(32)	100.0	196	-	-	3.6	88.4	8.0 A-C

Table 1. Continued

Replicates	Days post infection	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae
				23° C					
	10	50(40)	80.0	176	100.0	-	-	-	-
	18	35(27)	75.0	191	-	56.0	-	-	43.9 G-S
	19	35(30)	97.0	183	-	97.0	-	-	2.9 G-S
A	23	30(21)	70.0	47	-	10.0	89.9	-	-
	25	20(20)	100.0	94	-	-	100.0	-	-
	32	20(16)	80.0	48	-	6.2	30.0	30.0	33.7 A-C
	37	25(22)	88.0	86	-	2.0	25.0	65.0	7.9 A-C
	10	50(32)	64.0	113	100.0	-	-	-	-
	18	35(26)	74.2	257	3.0	77.0	-	-	20.9 G-S
	19	40(33)	82.5	115	2.1	87.8	-	-	10.0 G-S
B	23	30(23)	76.6	112	-	24.1	75.8	-	-
	25	25(19)	76.0	28	-	-	100.0	-	-
	32	20(20)	100.0	52	-	11.5	28.8	32.6	26.9 A-C
	37	30(18)	60.0	122	-	-	17.2	60.6	22.1 A-C
	10	40(32)	80.0	288	100.0	-	-	-	-
	18	35(29)	82.8	123	-	56.9	-	-	43.0 G-S
	19	35(26)	74.2	76	-	71.0	-	-	28.9 G-S
C	23	35(35)	100.0	119	-	24.3	75.6	-	-
	25	30(22)	73.3	148	-	13.5	86.4	-	-
	32	30(27)	90.0	150	-	-	25.5	22.7	51.7 A-C
	35	30(30)	100.0	197	-	-	9.1	72.5	18.3 A-C

Table 1. Continued

Replicates	Days post infection	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae
				23° C					
	10	50(35)	70.0	283	93.4	-	-	-	6.5 A
	18	40(40)	100.0	213	38.2	47.5	-	-	14.2 G-S
	19	35(20)	57.1	160	-	91.2	-	-	8.7 G-S
D	23	35(27)	77.1	214	-	14.4	85.5	-	-
	25	35(31)	88.5	132	-	12.1	87.8	-	-
	32	35(35)	100.0	206	-	-	13.5	18.4	67.9 A-C
	35	40(32)	80.0	194	-	-	6.7	71.1	22.1 A-C
	10	50(36)	72.0	275	100.0	-	-	-	-
	18	40(29)	72.5	178	19.1	75.2	-	-	5.6 G-S
	19	35(26)	74.2	248	-	75.1	-	-	24.8 G-S
E	23	35(31)	88.5	170	-	27.6	72.3	-	-
	25	35(23)	65.7	179	-	21.2	78.7	-	-
	32	40(38)	95.0	178	-	-	14.6	26.4	58.9 A-C
	35	40(38)	95.0	229	-	-	-	81.2	18.7 A-C
				25° C					
	10	50(35)	70.0	244	100.0	-	-	-	-
	15	40(27)	67.5	180	-	11.1	-	-	88.8 G-S
	18	40(32)	80.0	268	4.7	80.8	-	-	14.3 G-S
A	21	35(28)	80.0	76	-	18.4	81.5	-	-
	26	30(26)	86.6	110	-	-	10.2	-	89.7 A-C
	32	36(36)	100.0	131	-	6.5	11.5	81.8	-

Table 1. Continued

Replicates	Days post infection	Gammarids examined/infected	Percent infection	Number of larvae	1	2	3	4	Percent of unclassified larvae
				25° C					
B	8	50(35)	70.0	227	100.0	-	-	-	-
	15	50(31)	62.0	200	11.2	19.6	-	-	69.0 G-S
	17	40(37)	92.5	264	4.5	91.6	-	-	3.7 G-S
	21	40(40)	100.0	276	-	15.2	84.7	-	-
	26	50(37)	74.0	240	-	-	74.8	9.3	15.8 A-C
	32	47(41)	87.2	262	-	-	4.1	90.4	5.3 A-C
C	8	50(32)	64.0	161	100.0	-	-	-	-
	15	50(45)	90.0	201	7.4	8.9	-	-	83.5 G-S
	17	40(40)	100.0	202	-	82.1	-	-	17.8 G-S
	21	40(36)	90.0	178	-	-	95.5	-	4.4 G-S
	26	40(27)	67.5	225	-	10.6	24.0	2.6	62.6 A-C
	32	41(40)	97.5	251	-	7.9	13.1	70.9	7.9 A-C
D	9	50(46)	92.0	380	100.0	-	-	-	-
	15	50(33)	66.0	247	12.2	4.5	-	-	83.1 G-S
	19	40(29)	72.5	193	13.4	78.7	-	-	7.7 G-S
	21	40(33)	82.5	205	-	7.3	92.6	-	-
	26	40(29)	72.5	192	-	-	3.6	7.8	88.5 A-C
	32	53(51)	96.2	315	-	2.9	5.6	88.8	2.6 A-C
E	9	50(35)	70.0	155	100.0	-	-	-	-
	15	50(41)	82.0	289	11.2	37.7	-	-	51.0 G-S
	17	50(48)	98.0	218	12.0	83.6	-	-	4.3 G-S
	21	40(28)	70.0	167	5.1	9.1	85.6	-	-
	26	40(29)	72.5	329	-	3.6	31.6	7.2	57.4 A-C
	32	37(37)	100.0	136	-	-	-	100.0	-

Table 1. Continued

Note: A = acanthor

G-S = acanthella in G-stage as described for P. minutus by Hynes and Nicholas (1957)

A-C = acanthella-cystacanth as described for P. minutus by Hynes and Nicholas (1957)

APPENDIX V

Table 1a. Summarized raw data on the duration of the development of the larval stages of Polymorphus marilis at alternating temperatures (third experiment).

Replicates	Days post infection	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage				Percent of unclassified larvae
					1	2	3	4	
			15° C[a] - 25° C						
	16	40(29)	72.5	103	100.0	-	-	-	-
	25	40(33)	82.5	198	4.8	58.6	-	-	36.5 G-S
	28	40(36)	90.0	153	-	44.2	28.8	-	26.9 G-S
A	32	35(28)	80.0	219	-	9.1	87.2	-	-
	36	35(32)	91.4	116	-	0	100.0	-	-
	42	30(28)	93.3	114	-	8.7	43.3	47.7	-
	44	25(25)	100.0	273	-	-	18.8	78.3	30.5 A-C
	14	50(41)	82.0	191	100.0	-	-	-	-
	23	40(37)	92.5	325	-	86.3	-	-	13.6 G-S
	28	35(31)	88.5	105	-	71.4	28.5	-	-
B	30	35(28)	80.0	140	-	6.4	90.0	-	3.5 G-S
	36	40(38)	95.0	152	-	7.2	92.7	-	-
	42	30(29)	90.0	191	-	-	19.3	35.6	45.0 A-C
	44	29(23)	79.3	162	-	-	1.8	98.1	-
	14	50(35)	70.0	181	100.0	-	-	-	-
	23	50(48)	98.0	362	-	78.7	-	-	21.2 G-S
	28	40(40)	100.0	156	-	94.8	5.1	-	-
C	30	30(25)	83.3	89	-	14.6	85.3	-	-
	36	30(29)	96.6	150	-	7.4	92.5	-	-
	42	35(34)	97.1	193	-	-	10.3	41.6	50.7 A-C
	44	33(29)	87.8	122	-	-	5.7	94.2	5.7 A-C

Table 1a. Continued

Replicates	Days post infection	Gammarids examined/infected	Percent infection	Percent of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae
			15° C[a] - 25° C						
	12	50(36)	72.0	156	100.0	-	-	-	-
	21	40(31)	77.5	141	3.5	93.6	-	-	2.8 G-S
	28	35(29)	82.8	101	-	76.2	23.7	-	-
D	35	35(33)	94.2	193	-	19.8	71.7	-	8.3 G-S
	36	30(29)	90.0	106	-	-	100.0	-	-
	42	30(26)	86.6	202	-	-	10.8	19.3	69.8 A-C
	45	38(38)	100.0	436	-	2.9	7.0	90.0	-
			25° C[a] - 15° C						
	14	50(37)	74.0	181	100.0	-	-	-	-
	25	40(28)	70.0	150	7.7	76.7	-	-	15.5 G-S
	28	40(33)	82.5	131	-	70.0	29.0	-	-
A	35	30(26)	86.6	178	-	26.5	71.8	-	1.6 G-S
	36	30(28)	93.3	243	-	-	100.0	-	-
	42	35(31)	88.5	215	-	-	15.5	14.7	-
	47	37(29)	78.3	193	-	-	8.2	83.4	8.2 A-C
	17	50(35)	70.0	100	100.0	-	-	-	-
	23	40(40)	100.0	166	2.4	93.4	-	-	4.1 G-S
	28	35(26)	74.2	260	-	70.0	30.0	-	-
B	32	35(31)	88.5	303	-	16.3	68.6	-	15.0 G-S
	36	35(34)	97.1	188	-	2.2	81.8	-	15.9 G-S
	42	40(38)	95.0	184	-	-	28.2	11.9	59.7 A-C
	46	31(28)	90.3	251	-	2.6	8.9	88.3	-

Table 1a. Continued

Replicates	Days post infection	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage				Percent of unclassified larvae
					1	2	3	4	
C	13	50(32)	64.0	121	100.0	-	-	-	-
	26	40(38)	95.0	231	6.4	77.4	-	-	16.0 G-S
	28	35(32)	91.4	156	-	85.2	14.7	-	-
	34	35(31)	88.5	223	-	42.1	57.8	-	-
	36	35(28)	80.0	142	-	7.8	92.1	-	-
	42	30(29)	90.0	160	-	-	21.2	29.0	49.6 A-C
	46	39(39)	100.0	122	-	-	-	94.2	5.7 A-C
D	14	50(50)	100.0	253	100.0	-	-	-	-
	26	50(43)	86.0	187	-	89.3	-	-	10.6 G-S
	28	50(33)	66.0	176	-	89.7	10.2	-	-
	32	30(29)	90.0	194	-	18.5	71.1	-	10.3 G-S
	36	30(24)	80.0	235	-	15.7	84.2	-	-
	42	35(31)	88.5	121	-	-	8.2	4.1	87.6 A-C
	46	25(25)	100.0	129	-	-	3.2	87.9	8.8 A-C

[a]Temperature of first exposure

Note: G-S = acanthella in G-stage as described for P. minutus by Hynes and Nicholas (1957)

A-C = acanthella-cystacanth as described for P. minutus by Hynes and Nicholas (1957)

Table 1b. Summarized raw data on the duration of the development of the larval stages of Polymorphus marilis at alternating temperatures (third experiment).

Replicates	Total number of days at 23° C - 5° C	Days at 23° C	Days at 5° C	Gammarids examined/ infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae	
	21	11.0	10.0	50(37)	74.0	121	100.0	-	-	-	-	
	34	18.0	16.0	40(34)	85.0	218	9.4	60.5	-	-	30.0	G-S
A	46	24.0	22.0	40(31)	77.5	180	-	22.2	66.6	-	11.1	G-S
	64	33.0	31.0	35(29)	82.8	100	-	-	21.0	33.0	46.0	A-C
	73	37.0	36.0	69(63)	91.3	301	-	-	-	71.0	27.9	A-C
	21	12.0	9.0	50(42)	84.0	103	100.0	-	-	-	-	
	29	15.0	14.0	40(40)	100.0	198	31.3	4.5	-	-	64.1	G-S
	37	19.0	18.0	35(28)	80.0	298	-	83.5	-	-	16.4	G-S
B	46	24.0	21.0	30(29)	96.6	200	-	22.0	68.0	-	10.0	G-S
	48	25.0	23.0	40(36)	90.0	305	-	12.4	87.5	-	-	
	63	32.0	31.0	40(29)	72.5	160	-	-	50.0	25.0	25.0	A-C
	74	37.0	37.0	20(20)	100.0	86	-	-	18.6	72.0	9.3	A-C
	21	10.0	11.0	50(43)	86.0	233	100.0	-	-	-	-	
	31	16.0	15.0	40(38)	95.0	297	24.9	25.5	-	-	49.4	G-S
	35	18.0	17.0	30(27)	90.0	200	8.9	77.5	-	-	13.5	G-S
C	40	21.0	19.0	30(24)	80.0	99	-	55.5	11.1	-	33.3	G-S
	47	24.0	23.0	30(28)	93.3	172	-	-	72.0	-	27.9	G-S
	63	32.0	31.0	54(39)	72.2	401	-	-	55.3	18.4	26.2	A-C
	72	35.0	37.0	35(33)	94.2	100	-	6.0	28.0	54.0	11.9	A-C

Table 1b. Continued

Replicates	Total number of days at 23° C - 5° C	Days at 23° C	Days at 5° C	Gammarids examined/ infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae	
D	21	11.0	10.0	40(31)	77.5	217	100.0	-	-	-	-	
	35	19.0	16.0	40(27)	67.5	300	27.3	59.0	-	-	13.6	G-S
	42	22.0	21.0	40(36)	90.0	298	10.4	33.2	56.3	-	-	
	46	23.0	23.0	30(23)	76.6	320	-	19.6	69.0	-	11.3	G-S
	63	32.0	31.0	30(19)	63.3	115	-	-	4.3	22.6	73.0	A-C
	64	37.0	37.0	41(41)	100.0	192	-	-	29.6	68.2	2.0	A-C

Note: G - S = acanthella in G-stage as described for P. minutus by Hynes and Nicholas (1957)

A - C = acanthella-cystacanth as described for P. minutus by Hynes and Nicholas (1957)

APPENDIX VI

Table 1. Summarized raw data on the duration of the stages of development of Polymorphus marilis in Gammarus lacustris after incubation at 10° C at varying periods, then transferred to 23° C (fifth experiment).

Replicates	Days at 23° C	Gammarids examined/infected	Percent infection	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae	
a. Development at 23° C after transfer from 10° C on day 28										
A	10	50(31)	62.0	280	100.0	0.0	-	-	-	
	23	50(41)	82.0	207	-	84.5	-	-	15.4	G-S
	35	50(29)	58.0	86	-	-	100.0	-	-	
	56	79(66)	83.5	147	-	-	23.1	76.8	-	
B	10	50(38)	76.0	250	100.0	-	-	-	-	
	23	50(43)	86.0	175	3.1	88.5	-	-	8.3	G-S
	35	50(39)	78.0	142	-	-	100.0	-	-	
	56	63(50)	79.3	256	-	-	9.0	82.9	9.0	A-C
C	10	50(46)	92.0	190	100.0	-	-	-	-	
	23	50(37)	74.0	192	5.6	84.3	-	-	11.0	G-S
	35	50(35)	70.0	126	2.6	10.0	87.3	-	-	
	56	56(47)	83.9	289	-	2.2	5.0	88.5	4.1	A-C

Table 1. Continued

Replicates	Days at 23° C	Gammarids examined/infected	Percent infected	Number of larvae	Percent larvae at each stage 1	2	3	4	Percent of unclassified larvae
b. Development at 23° C after transfer from 10° C on day 40									
A	10	50(50)	100.0	372	-	88.0	11.9	-	-
	12	50(44)	88.0	226	-	22.1	77.8	-	-
	20	66(58)	87.8	402	-	-	26.4	73.5	-
B	9	50(41)	82.0	270	-	89.1	10.8	-	-
	12	50(41)	82.0	263	-	20.5	79.5	-	-
	21	92(67)	72.8	197	-	-	13.6	82.9	3.4 A-C
C	10	50(45)	90.0	199	-	77.8	22.1	-	-
	13	50(33)	66.0	172	-	8.7	91.3	-	-
	23	60(49)	81.6	264	-	6.4	7.9	85.6	-

Note: G-S = acanthella in G-stage as described for P. minutus by Hynes and Nicholas (1957).

A-C = acanthella-cystacanth as described for P. minutus by Hynes and Nicholas (1957).